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TITLE: Prediction of Future Disability in MS Using Combined Novel MRI and Serological Markers

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14. ABSTRACT
In this annual report, we detailed our activities in the dates since initial IRB approval (10/26/2019)with emphasis on activities since our last annual report. We have continued to contact and schedule patients for study visits, beginning chart review for patients who prefer to come later for their study visit. 38 patients have completed study visits, 20 are scheduled for upcoming visits, and 96 patients have been contacted overall. MRI data reprocessing has been completed for all subjects. Study visits are ongoing and chart reviews have been started.

15. SUBJECT TERMS
None listed.

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1. INTRODUCTION:

We will study novel MRI and serological markers (analyzed on previously collected imaging and serum samples) as predictors of future disability in Multiple Sclerosis (MS). MS is heterogenous, patients display a wide spectrum of long-term disability levels that are not completely foreseen by early disease activity but may be explained, in part, by intrinsic patient-specific differences in central nervous system tissue susceptibilityto damage and intrinsic ability to repair. Novel MRI and serological biomarkers, such as Gradient Echo Plural Contrast Imaging (GEPCI) and serum neurofilament light chains (NfL), may better reflect the early neurodegeneration in MS. We wish to determine if these novel biomarkers, separately and in combination, can be used as better predictors of disability in individual patients and can ultimately guide treatment choices. We identified 127 well-described subjects from four prior GEPCI studies that have at least one brain MRI with GEPCI data acquired at 3.0 Tesla. All 127 will be recruited to a single follow-up visit, in which each subject will undergo testing by a blinded examiner, with Expanded Disability Status Scale (EDSS), MS Functional Composite (MSFC) and Symbol Digit Modality Test (SDMT) and Montreal Cognitive Assessment (MoCA). MRI images will be re-analyzed for R2t* and Quantitative susceptibility maps (QSM). We will calculate average R2t* values from cortical and deep gray matter (GM), normal-appearing white matter and lesions, and determine individual regional patterns of CNS damage. We will also derive QSM from GEPCI to evaluate lesions and deep GM degeneration. In stored serum samples, we will measure serum NfL by single molecule array (Simoa). Associations of MRI changes and NfL levels with rate of disability accumulation will be determined. We all also build a multivariate model that best predicts future disability.

2. KEYWORDS:

Multiple Sclerosis, Biomarkers, Gradient Echo MRI, Neurofilament light chain, disability, prediction

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The major goals of the project were as follows:

Major Task 1: Preparation to begin the study (100% completion of this goal was met on 5/14/2020)

a) Prepare IRB and HRPO submission, including informed consent, completed 10/26/2019.
b) Finalize consent form and human subjects protocol, completed 5/14/2019
c) Prepare Data entry capture forms in REDCAP, completed 5/14/2020
d) Train investigators and research coordinator in REDCAP data entry, completed 5/14/2020
e) Milestone: Completion of IRB and HRPO review and consent process. Completion of RedCap data elements. Milestone met 5/14/2020

Major Task 2: Follow-up visits

a) Subtask 1: Ensure that clinical investigators are trained in Expanded Disability Status Scale (EDSS). 100% completed 10/26/2019
b) Subtask 2: Ensure that research coordinator is trained in administration of Multiple Sclerosis Functional Composite (MSFC), Symbol Digit Modalities Test (SDMT). 100% completed 10/26/2019
c) Subtask 3: Contact and evaluate study subjects (n=127, relapsing remitting MS (RRMS)=60, secondary progressive MS (SPMS)=43 and primary progressive MS (PPMS)=24). Subtask is at 45% completion
d) Milestone: Follow-up visits completed (n=127). 30% completed

Major Task 3: Image analysis

a) Download GEPCI data and prepare for analysis (n=127), one scan per MS patient. 100% completed

b) Apply new processing to older GEPCI images (to obtain updated R2t* values), perform quality control on old and new images, 100% completed on 9/30/2021

c) Re-analysis of GEPCI images to obtain R2t* values in all brain areas. 40% completed

d) Generating GEPCI-Barcode areas and values. 0% completed

e) Generating Quantitative Susceptibility maps (QSM) data. 0% completed

f) Milestone: Completion of MRI re-analysis. 0% completed

Major Task 4: Serum analysis

a) Subtask 1: Purchase NfL assay, prepare for Single molecule array (Simoa) analysis. 0% completed.

b) Subtask 2: Coordinate timing of equipment use with Holtzman laboratory and ensure NfL assay is working accurately. 0% completed

c) Subtask 3: Analysis of samples from study subjects (n=88). One serum sample for each patient for a total of 88 samples. 0% completed

d) Milestone: Completion of serum analysis (n=88). 0% completed

Major Task 5: Data organization and analysis

a) Subtask 1: Obtain and organize previously collected data on study subjects (and enter in REDCAP). 50% completed

b) Subtask 2: Descriptive and summary statistics. 0% completed

c) Subtask 3: Study the association between MRI variables and future disability. 0% completed

d) Subtask 4: Study the association between serum NfL levels and future disability. 0% completed

e) Milestone: Complete initial data analysis. 0% completed

Major Task 6: Develop and test a multivariate model for the prediction of disability in MS.

a) Subtask 1: Construct a multivariate linear model for disability prediction (that incorporates MRI, serum makers and other important disease and patient variables). 0% completed

b) Subtask 2: Validate the multivariate model using a cross-validation technique. 0% completed

c) Subtask 3: Review study results, statistical analysis output and discuss results with study team. 0% completed

d) Milestone: Complete data analysis and study. 0% completed

What was accomplished under these goals?

For the first Major Task, 100% completion of this goal was achieved 5/14/2020. Under these goal initial materials, including the finalized consent form, were submitted to the Washington University IRB on 5/14/2019 and received approval on 10/26/2019. Data capture forms were developed for the project REDCAP database, which was moved from development into production on 5/14/2020. Investigators and study coordinators were trained on the entry of study data into the REDCAP database.

For the second major task, the training of clinical investigators and coordinators for the administration of their allocation study assessments was confirmed to be complete when IRB approval was received for this project. Participants with the oldest GEPCI MRIs were prioritized when participant contact began. For patients who are unable to be seen for a study visit in conjunction with a clinical appointment, with their permission via phone consent the chart review process is started with plans to complete an in-person study visit later. As of 10/11/2021, study coordinators have contacted 96 patients, 28 participants have completed an in-person study visit, 10 chart reviews visits have been completed under the consent waiver and 20 have upcoming visits scheduled over the next four months. Yassin Mryeoud, a research analyst trained in chart review, has started reviewing the charts of consented subjects (or, when applicable, under waiver of consent).

For the third major task, MRI images for all 127 study participants have been searched for, located and downloaded (if available).
New processing was applied to older GEPCI images to obtain updated R2t* values. Quality control was applied to both old and new images. For some of the older GEPCI images (from the CombiRx study), additional anatomical images were located to aid in the processing and segmentation of the GEPCI data. Out of 127 subject/scans, 16 cannot be included in this study (due to not having any usable GEPCI images or good quality anatomical images or due to significant artifact). Thus 111 images were re-processed (if needed) and made ready for analysis. For subjects with more than one GEPCI MRI, the oldest scan with good quality images was used (to allow a larger follow-up time).
Scan from 48 subjects of (43% available MRI, 38% of all subjects) were analyzed. Those working with the imaging data are blinded to the clinical outcomes of the subjects. Reconstructed GEPCI scans were imported into MATLAB for further processing. We used an in-house MATLAB script developed by the Dr. Yablonskiy group to carry out further processing. For processing, first a Hanning filter was applied to correct for image artifacts. Then using a FSL Brain extraction tool we stripped the skull and extracted the brain from the images. Calculations for frequency mapping were run, then frequency unwrapping and frequency extension were performed. To complete processing, the first BOLD procedure calculation was completed. After completing the processing pipeline, we obtain the primary GPCI images, including 't1w', 'R2t', 'fre', 'dfre', 'Kesai', 'Ksi'. The resulting R2t maps were converted to NIFTI format and visually inspected for image artifacts.

We plan to run the serum analysis in the early part of 2022, we will purchase the assays and run the testing at that time.

For the fifth major task, data is being collected and organized through study review and chart review for input into the Redcap database.

The sixth major task is dependent on the completion of the previous five tasks and is not yet started.

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

In the next reporting period, we plan to continue to contact participants and conduct study visits. We also plan to continue the chart review process, consolidating and validating data from subjects, previously collected study data and chart review into the current Redcap database. Chart review will proceed for subjects who have consented for the study (i.e. before or during the study visit), those who are unable to attend a study visit or those who cannot be reached (as detailed in our IRB application).
New imaging processing and quality control is complete. The MRI images are all downloaded and ready to be analyzed. Data has been generated from 48 scans. The rest of the scans will be analyzed in the spring of 2022 in preparation for full study analysis. Serum analysis will also occur in the spring of 2022.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

We continue to experience some delay in our ability to schedule and perform on-site visits because of University-wide restrictions on in-person visits and participant hesitancy to come in for a research study visit during the ongoing pandemic. As described in our previous annual reports, we are performing chart reviews ahead of scheduled in-person visits.

Actual or anticipated problems or delays and actions or plans to resolve them

Our plan to address the challenges we are experiencing due to the COVID-19 pandemic remains the same: we modified our chart review procedures to be able to collect data prior to the completion of an in-person study visit and prioritizing participants who would likely only complete assessments by phone due to mobility or disease related limitations. For patients who scheduled for in-person clinic visit, we offering to complete their study visit on the same day to minimize the need for additional trips to our site.

Changes that had a significant impact on expenditures

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS:

Publications, conference papers, and presentations

Nothing to Report.

Journal publications.

Nothing to Report.

Books or other non-periodical, one-time publications.

Nothing to Report.

Other publications, conference papers and presentations.

Nothing to Report.

Website(s) or other Internet site(s)

Nothing to Report.

Technologies or techniques

Nothing to Report.

Inventions, patent applications, and/or licenses

Nothing to Report.

Other Products

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name	Dorothy Anne Cross
Project Role	Principal Investigator
Nearest person month worked	0.96
Contribution to Project	Dr. Cross has overseen the overall study, the build of the REDCap database, and performed study visits.
Funding Support	(effort supported by this grant)

Name	Amber Salter
Project Role	Co-Investigator
Nearest person month worked	1.2
Contribution to Project	Dr. Salter has contributed to the build of the REDCap database. Role with Washington University ended in **May 31, 2021**
Funding Support	(effort supported by this grant) Role with Washington University ended in **May 31, 2021**

Name	Dmitriy Yablonskiy
Project Role	Co-Investigator
Nearest person month worked	1.08
Contribution to Project	Dr. Yablonskiy has managed the MRI analysis and image quality control.
Funding Support	(effort supported by this grant)

Name	Biao Xiang
Project Role	Co-Investigator
Nearest person month worked	2
Contribution to Project	Dr. Xiang has contributed the analysis and procurement of MRI images for the study.
Funding Support	Effort supported from this grant through 6/30/2020, supported 100% from the National Multiple Sclerosis (MS) Society **beginning 7/1/2020**.

Name	Sayan Kahali
Project Role	Co-Investigator
Nearest person month worked	4
Contribution to Project	Dr. Kahali has contributed the analysis and procurement of MRI images for the study.
Funding Support	Work (effort) on this project supported by this grant (Dr. Kahali started on 7/1/2020)

Name	Salim Chahin
Project Role	Co-Investigator
Nearest person month worked	4.8
Contribution to Project	Dr. Chahin has overseen the overall study and the build of the REDCap database. He has performed study visits and trained study team members on data extraction during chart review, REDCap data entry and the cognitive and functional assessments for this study.
Funding Support	(effort supported by this grant)

Name	Amjad Samara
Project Role	Research Analyst
Nearest person month worked	0.60
Contribution to Project	Dr. Samara has contributed to the MRI analysis. Role ended on 6/30/2021
Funding Support	National Institute on Drug Abuse

Name	Alyssa Spurling
Project Role	Research Coordinator
Nearest person month worked	2.4
Contribution to Project	Mrs. Spurling has organized the subjects list, identified clinical MRIs proximal to GEPCI MRI, organized additional patient data, and contacted study subjects. Role in this project ended on 10/1/2021
Funding Support	(effort supported by this grant) – ended on 10/1/2021

Name	Courtney Dula
Project Role	Research Coordinator
Nearest person month worked	2.4
Contribution to Project	Mrs. Dula has organized the subjects list, organized additional patient data, contacted study subjects, performed the study visits and help with

	the quarterly and annual reports.
Funding Support	(effort supported by this grant)

Name	Yassin Mryeoud
Project Role	Research Analyst
Nearest person month worked	0.2
Contribution to Project	Mr. Mryeoud is completing the chart review for enrolled study participants.
Funding Support	Effort is supported by Washington University Department of Neurology

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

See below and see attached for updated support documents.
Dr. Amber Salter has relocated from Washington University in May 2021. Her role in this study will not be filled immediately. We will plan to ask for bio statistical consultation from within Washington University when the study visits are complete and study analysis is needed (we will thus pay only for a one time consultation fee and not an ongoing effort sourcing).

Alyssa Spurling, study coordinator, has transitioned off this project as of 10/1/2021.

Yassin Mryeoud has joined our team to work on the chart review process.

Anne Cross (Principal Investigator)

Project(s) Ended:

"Discontinuation of Disease Modifying Therapies (DMTS) In Multiple Sclerosis" CER150327915. **Ended 7/31/2021**

Project(s) Started:

"Using quantitative gradient echo MRI to distinguish MOG antibody disorder from multiple sclerosis" NIH: R03NS114028. **Started 4/1/2021**

"CHIMES: Prospective Study to Assess Disease Activity and Biomarkers in Minority Participants With Relapsing Multiple Sclerosis (RMS) After Initiation and During Treatment With Ocrelizumab" Genentech. ClinicalTrials.gov: NCT04377555. **Started 5/1/2021**

Dmitriy Yablonskiy (Co-Investigator)

Project(s) Ended:

"Gradient Echo MRI to Detect and Measure Evolution of Progressive MS" Conrad N. Hilton Foundation. 20140257. **Ended 12/31/2020**

Project(s) Started:

Nothing to report

Salim Chahin (Co-Investigator)

Project(s) Ended:

"Novel Multiple Sclerosis Biomarkers" The Barnes-Jewish Foundation. **Ended 10/20/2020**

"COVID-19 Risk in Multiple Sclerosis (Survey)" Biogen. **Ended 12/31/2020**

Project(s) Started:

Nothing to report

Biao Xiang (Co-Investigator)

Project(s) Ended:

"Gradient Echo MRI to Detect and Measure Evolution of Progressive MS" Conrad N. Hilton Foundation. 20140257. **Ended 12/31/2020**

Project(s) Started:

Nothing to report

Sayan Kahali (Co-Investigator)

Project(s) Ended:

Nothing to report

Project(s) Started:

Nothing to report

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Nothing to Report.

QUAD CHARTS:

Nothing to Report.

9. APPENDICES: