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TITLE: Establishing Network Connectivity and Microvascular Imaging Biomarkers for Tuberous Sclerosis Complex

PRINCIPAL INVESTIGATOR: Dr. Mark DiFrancesco, PhD

CONTRACTING ORGANIZATION: Children's Hospital, Cincinnati

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6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Cincinnati Children's Hospital
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14. ABSTRACT

Background: Tuberous Sclerosis Complex (TSC) often results in the development of tubers that vary widely among patients in number, size, and location. Often, the presence of these tubers results in seizures, but epilepsy phenotype can vary with no clear association with number of tubers. The challenge for TSC surgical treatment is the usual presence of multiple tubers. There is clear need for tuber biomarkers that can identifyand potentially predict epilepsy phenotype and cognitive outcomes. This project applies advanced imaging techniques to extract functional network connectivity features of tuber arrays in patients that associate with epilepsy and cognitive phenotypes. Importantly, the project will also use imaging to ascertain microvascular density and topology from individual tubers to help distinguish those that impact seizure activity and cognition.
Hypotheses: 1) epilepsy phenotype and cognitive outcomes are associated with signatures of disruption of canonical networks in the brains of TSC patients, 2) the intrinsic connectivity of the brain regions occupied by tubers correspond to phenotype and outcomes, and 3) neurovascular properties of tubers will differ from healthy tissue and serve as complementary physiologic features of individual tubers that associate with phenotype and cognitive outcomes.
Specific Aim 1: Determine MRI functional connectivity signatures among TSC patients that distinguish epilepsy phenotype and associate with cognitive performance. **Specific Aim 2:** Determine deviations of brain tissue microvascular density in and around individual tubers compared to healthy tissue and assess the contribution of individual tubers to patient network connectivity. **Specific Aim 3** Associate individual tuber region connectivity pattern coupled with microvascular density with clinical suspicion of epileptogenicity via stereoEEG (sEEG) in a subset of TSC patients. **Study Design:** TSC patients (n=100, age 2-10 years) of different epilepsy phenotype will be scanned by resting-state fMRI and a diffusion-weighted imaging series for intravoxel incoherent motion modeling appended to standard clinical MRI. They will be scanned a second time after 1 year.

15. SUBJECT TERMS
Tuberous Sclerosis Complex, MRI, functional connectivity, epilepsy, cognition, brain microvasculature, brain microstructure, tubers, lesion network mapping

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1. INTRODUCTION:

Tuberous Sclerosis Complex (TSC) often results in the development of regions of cortical and subcortical focal cortical dysplasia (tubers) that vary widely among patients in number, size, and location. Often, the presence of these tubers results in seizures, but again, the epilepsy phenotype (no epilepsy, drug-sensitive epilepsy, or drug-resistant epilepsy) can vary among patients. The number of tubers has no clear association with phenotype. The challenge for TSC surgical treatment is the usual presence of multiple spatially scattered tubers. There is clear need for tuber biomarkers that can identify, track, and potentially predict epilepsy phenotype and cognitive outcomes. This project applies advanced imaging techniques to extract functional network connectivity features of tuber arrays in patients that associate with epilepsy and cognitive phenotypes. Importantly, the project will also use imaging to ascertain microvascular density and topology metrics from individual tubers to help distinguish those that impact seizure activity and cognitive performance. We aim to 1) determine MRI functional connectivity signatures among TSC patients that distinguish epilepsy phenotype and associate with cognitive performance, 2) determine deviations of brain tissue microvascular density in and around individual tubers compared to healthy tissue and assess the contribution of individual tubers to patient network connectivity that distinguish epilepsy phenotype and associate with cognitive performance, and 3) associate individual tuber region connectivity pattern coupled with microvascular density with clinical suspicion of epileptogenicity via stereoEEG (sEEG) in a subset of TSC patients.

2. KEYWORDS:

Tuberous Sclerosis Complex, MRI, functional connectivity, epilepsy, cognition, brain microvasculature, brain microstructure, tubers, lesion network mapping

3. ACCOMPLISHMENTS:

What were the major goals of the project?

1. IRB and HRPO approval. Milestone: 3 months
    a. IRB approved: 5 July 2022.
    b. HRPO approved: 29 August 2022.
2. Set up imaging protocol. Milestone: 3 months
    a. Optimize protocol on research scanner: complete October 2022
    b. Install protocol on clinical scanner:
        i. Philips clinical key necessary to run protocol was obtained 14 November 2022.
        ii. Dry run with human volunteer completed 22 November 2022.
        iii. Protocol is complete and ready, including formal instructions for MRI technicians.
3. Acquire imaging and cognitive testing data. Milestone 36 months
    a. Acquire imaging and cognitive testing data as part of TSC clinical visits (2-24 months): By this time, SOW indicates we should have more than 50 acquisitions. We have completed 4 as of the end of this reporting period. We currently have nearly 50 patients in the pipeline for enrollment. Two patients are scheduled during the next week. The scheduling pipeline with Radiology is working well. We continue to adjust the recruitment strategy to capture patients using a longer time horizon so that families can be introduced to the study well before clinical visits during which they would undergo a clinical MRI. We are also sharing recruitment roles with another TSC-related study at our site.
    b. Acquire 1-year follow-up data (13-36) months. Not yet started.
4. Analyze data. Milestone 36 months.
    a. Analyze resting-state and DWI data (6-36 months): Not yet started.
5. Analyze DRE patient data for individual tubers assessed by SEEG in preparation for surgery. Milestone 36 months, starting at 25 months. Not yet started
6. Prepare manuscripts and submit for publication. Milestone 36 months. Not yet started.

What was accomplished under these goals?

1. Major activities: Complete setup of research protocol to append to clinical MRI. This included acquisition of a clinical key from Philips to allow the research sequences. In addition, we performed testing of the protocol with a volunteer. Formal instructions for MRI technicians were produced.
2. Data acquisitions have started. A recruitment and scheduling pipeline is in place.

According to SOW, we expected to have acquired data on more than 50 patients by this period. We have completed 4 by July 15, 2013. Two more patients are scheduled for the next week and more nearly 50 patients are in the pipeline. Strategies are being discussed in ongoing monthly team meetings to better capture patients eligible for the study in anticipation of clinical visits during which MRI will be performed as part of SOC. We are also always working with Radiology and Anesthesiology to better coordinate with TSC patient visits. Though we as still behind in recruitment, processes are in place and continue to be developed to fill the gap.

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

The project was not intended to provide training or professional development. However, as appropriate, opportunities to provide mentorship and support to interested early-stage researchers would be welcomed.

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?

As stated above, the main effort currently is to maintain and accelerate recruitment and data acquisition. We will continue to streamline the contact, scheduling, and acquisition protocols. We are also partnering with other studies related to TSC at our site to improve awareness of our study and to potentially find recruits.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

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

Since we are still in the beginning stages of recruitment and data acquisition, there is nothing yet to report regarding impact of findings.
Nevertheless, we have developed a research imaging protocol for appending to clinical protocols, geared toward lesion network mapping, that includes state of the art resting-state fMRI with multi-echo acquisition, DWI at multiple b-values, and the addition of synthetic MRI sequence for obtaining relaxometry metrics.

What was the impact on other disciplines?

There are no findings yet. It is anticipated that techniques developed here for TSC will apply to other conditions impacted by focal lesions.

What was the impact on technology transfer?

Nothing to report at this time.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

There were no significant changes in approach.

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

The project is behind for recruitment and acquisitions. Since the start of the project, we have had patients in the pipeline, but their clinical MRI visits were often scheduled weeks or months into the future. We continue to work on strategies to allow a longer time window for patient contact so that clinical MRI can be scheduled with the research add-on for the next clinical visit by the patient. Otherwise, patients may be contacted only after a recent MRI had already been done. We have also implemented a process by which some patients could do the research MRI and the neurocognitive testing on separate visits. As mentioned to the science officer in a recent correspondence, we are also sharing some neurocognitive testing data with another TSC-related study being conducted at our site. This will facilitate the acquisition of cognitive testing for our study. These measures mitigate the challenge of scheduling MRI and neuropsychological testing on the same visit. We are also always working with Radiology and Anesthesiology to better coordinate sedated scan scheduling with typical TSC clinic visits.
Scanning of patients for the study has now begun and additional patients are scheduled. We anticipate that ongoing strategic approaches will allow recruitment of patients and scheduling of research MRI add-ons in a timely manner.

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

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Not applicable.

Significant changes in use of biohazards and/or select agents

Not applicable.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**

  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**

  A research imaging protocol for appending to clinical protocols, geared toward lesion network mapping, that includes state of the art resting-state fMRI with multi-echo acquisition, DWI at multiple b-values, and the addition of synthetic MRI sequence for obtaining relaxometry metrics. Not yet shared.

- **Inventions, patent applications, and/or licenses**

  Nothing to report.

- **Other Products**

  A research imaging protocol for appending to clinical protocols, geared toward lesion network mapping, that includes state of the art resting-state fMRI with multi-echo acquisition, DWI at multiple b-values, and the addition of synthetic MRI sequence for obtaining relaxometry metrics.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

1. Mark DiFrancesco
   - **a.** Role: PI
   - **b.** ID: 0000-0001-8002-0332
   - **c.** Person months: 1
   - **d.** Contributions: overseeing project as PI, setup and implementation of imaging protocol, study design.
2. Darcy Krueger
   - **a.** Role: co-investigator
   - **b.** ID: 0000-0002-7250-7391
   - **c.** Person months < 1
   - **d.** Contributions: study design, patient recruitment, medical consultation, TSC specialist. Note that Dr. Krueger's effort was reduced to 1% from 5%, as day to day activities related to this project will be performed more by Dr. Ritter, whose effort consequently increased by 4%.
3. Hans Greiner
   - **a.** Role: co-investigator
   - **b.** ID: 0000-0001-7479-1384
   - **c.** Person months < 1
   - **d.** Contributions: study design, patient recruitment, medical consultation, epilepsy specialist
4. David Ritter
   - **a.** Role: co-investigator
   - **b.** ID: 0000-0001-7723-3636
   - **c.** Person months: 1
   - **d.** Contributions: patient recruitment, medical consultation, patient screening. Please see note for Dr. Krueger above regarding a reallocation of effort.
5. Adrienne Victory
   - **a.** Role: research coordinator
   - **b.** ID: n/a
   - **c.** Person months < 1
   - **d.** Contributions: coordinator for recruitment, screening, and acquiring data
6. Madeleine Robben
   - **a.** Role: research coordinator
   - **b.** ID: n/a
   - **c.** Person months: 1
   - **d.** Contributions: coordinator for recruitment, screening, and acquiring data
7. Theresa Hennard
   - **a.** Role: regulatory
   - **b.** ID: n/a
   - **c.** Person months < 1
   - **d.** Contributions: regulatory preparation, IRB processes.
8. Anna Byars
   - **a.** Role: co-investigator: neuropsychiatric
   - **b.** ID: 0000-0002-3929-6107
   - **c.** Person months < 1
   - **d.** Contributions: set up and design of neuropsychiatric assessments
9. Paul Horn
   - **a.** Role: biostatistician
   - **b.** ID: n/a
   - **c.** Person months < 1
   - **d.** Contributions: consultation on statistical analysis

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

Mark DiFrancesco
Added: Lupus Research Alliance grant.
Title: Vascular Pathophysiology in CNS SLE: The Blood-CSF Barrier
PI: DiFrancesco
Goal: Advanced quantitative imaging methods will used to measure regional microstructural differences in the brain microvasculature in healthy controls and in MS and SLE subjects. Differences will be correlated with cognitive assessments in these subjects.
Period: 03/01/23 – 02/28/26.
Effort: 1.8 months.

Completed: Department of Defense: W81XWH180615
Title: Novel Neuroimaging Assessments of Glymphatic Disruption in Humans, a Plausible Key Pathophysiological Mechanism for CNS Lupus.
PI: DiFrancesco

Completed: NIH: R21DC017393
Title: Multimodal Neuroimaging Distinguishes Developmental and Disordered Phenotypes in Speech Sound Disorders.
PI: Vannest. Role: co-I.

David Ritter

Added: Department of Defence: HT94252310212
Title: Cardiac Rhabdomyomas as Biomarkers of TSC Disease Severity
PI: Ritter
Period: 07/2023 – 07/2025
Effort: 0.6 months.

Darcy Krueger and Anna Byars
Completed: NIH NINDS: 1U01NS092595-01A1
Title: Preventing Epilepsy Using Vigabatrin in Infants with Tuberous sclerosis
Major Goals: The central hypothesis of this Phase IIb trial is that early identification of electroencephalography (EEG) biomarkers and early treatment versus delayed treatment with vigabatrin in infants with tuberous sclerosis complex (TSC) will have a positive impact on developmental outcomes at 24 months of age. It would also prevent or lower the risk of developing infantile spasms and refractory seizures.
PI: Martina Bebin
Period: 06/2016 - 05/2023

Darcy Krueger
Completed: CHMC-CpG.
Title: Phenotype-Genotype relationships in Tuberous Sclerosis
Major Goals: To identify any genotypic-phenotypic relationships in patients with clinically-diagnosed TSC.
PI: Danzer, Steve.
Period: 07/2021 - 06/2023

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Not applicable.

QUAD CHARTS: Submitted with report.

9. APPENDICES: None.