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TITLE: Understanding the role of TSC1/2 in cerebellar Purkinje neurons

PRINCIPAL INVESTIGATOR: Mustafa Sahin

CONTRACTING ORGANIZATION:

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Boston, MA 02115-5724

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CHILDREN'S HOSPITAL BOSTON
THE OFFICE OF SPONSORED PROGRAMS
300 LONGWOOD AVE, BOSTON MA 02115-5724

8. PERFORMING ORGANIZATION REPORT NUMBER

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Tuberous sclerosis complex is a rare multisystem disorder caused by mutations in either TSC1 or TSC2 genes. Neurological manifestations of TSC include epilepsy, cortical tuber formation and increased prevalence of autism in the patients. Previously our group has shown that *Tsc1* knock-out mice Purkinje cells are involved in the development of autistic-like features for these mice. In this study we have developed differentiation protocol for generation of human Purkinje cells (PCs) from hiPSCs derived from TSC-patients with heterozygous TSC2-mutations. We have studied mTOR-pathway hyperactivation in TSC2-deficient patient hiPSC-derived PCs compared to control cells and and characterized the transcriptional profiles of the TSC2-deficient human PCs *in vitro*. We have also created an isogenic control hiPSC-lines with correction of the heterozygous TSC2-microdeletion in TSC-patient derived hiPSCs. To study TSC2-deficits in the isogenic human cells we have characterized the cerebellar precursor cell development and PC differentiation of the isogenic control hiPSC-clones in parallel with TSC2-deficient isogenic patient derived cells. Transcriptional profiling and characterization of the disease phenotypes of the TSC2 deficient hiPSC-derived PCs may discover interesting targets for the future development of pharmacotherapy for TSC-patients with autism.

15. SUBJECT TERMS

autism, tuberous sclerosis, cerebellum.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	15	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION:

Tuberous sclerosis complex (TSC) is a multisystem autosomal dominant disorder caused by mutations in either *TSC1* or *TSC2* genes. TSC patients are often affected with developmental delay and epilepsy and approximately half of patients with TSC display symptoms of autism spectrum disorder (ASD). Although much research has been conducted, the neural circuitry and molecular mechanism underlying autism remain unclear. Specific cerebellar defects have been seen in TSC patients, suggesting a crucial role for the cerebellum. Cerebellar pathology can be found in 1 in 3 patients with TSC, and studies correlate cerebellar pathology with ASD symptomatology in patients with TSC. Purkinje cells are the sole output neuron of the cerebellum, and previously we have shown that *Tsc1* mutant Purkinje cells cause autistic-like behaviors in mice. The objective of this study is to establish a novel platform to characterize *TSC1/2*-mutation related neurodevelopmental disorders and ASD related cellular dysfunctions in *in vitro* and *in vivo* models of human patient specific iPSC-derived Purkinje cells. The results of this study will provide important insights about the molecular mechanism underlying neurodevelopmental disorders. In the future, the results of this study can be used to establish novel therapeutic targets for treatment of TSC patients diagnosed with or at risk of developing ASD.

2. KEYWORDS: Human iPSC, TSC1/2, Purkinje cells, disease phenotyping, autism, cerebellum.

3. ACCOMPLISHMENTS:

Specific Aims:

Aim 1: To compare the transcriptional profiles of mouse and human PCs with and without TSC1/2 expression. As a first step, we will carry out RNAseq in PC's isolated from Tsc1-knockout mouse and control mice and compare the gene expression profiles to identify differences in expression of transcription factors during PC development. Using the protocol developed by the Hatten lab for differentiating hES cells into PCs, we will select iPSC clones that express the Pcp2 bacTRAP tag (*Pcp2-Egfp-L10a*), differentiate them into PCs and assay the transcriptomes of iPSC-derived PCs from TSC and unaffected patients. We will then use bioinformatics to identify gene pathways that differ in Tsc1 mutant mouse and human PCs compared with wild type/unaffected cells.

Aim 2: To genetically correct TSC mutations in patient-specific iPSC lines and rescue the disease phenotypes in patient specific neurons *in vitro*. We will employ CRISPR-Caspase 9 (Cas9) genome editing techniques to correct TSC1/2 mutations in patient-specific iPSC lines and evaluate the reversibility of any observed phenotypes. The TSC1/TSC2-deficient iPSCs will be differentiated into neuronal cells to establish comprehensive cellular phenotypic analysis compared to isogenic controls, including cell vulnerability to a range of insults, oxidative stress, and electrophysiological properties reflecting cellular excitability.

Aim 3: To evaluate functionality and neural circuit formation capacity of human patient derived cerebellar precursor and PCs *in vivo* in Tsc1-PC knockout mice. The goal is to characterize the functionality and differentiation capacity of human iPSC-derived cerebellar precursors and PCs *in vivo*. We will study if human TSC patient neurons and control neurons survive, and develop normally, integrate into the correct layer of the mouse cerebellum and show functionality. We will analyze dendritic arborization, axon outgrowth, and use electrophysiology to measure neurons excitability. If successful, this research will provide a critical new model system for analyzing disorders that involve human

cerebellar circuit development. This study will provide proof of concept that loss of PCs is responsible for the behavioral deficits in the Tsc-deficient animals.

Studies and Results

For Aim 1. In order to analyze gene expression specifically from Purkinje cells within a mixed culture of cells, a clonal Pcp2-EGFP-L10a TRAP-hESC line was generated using lentivirus. The EGFP-L10a TRAP tag is expressed both during initial differentiation and following isolation of PCs and co-culture with mouse granule cells (Figure 1). RNA isolated from PCs at various stages of differentiation are being assayed by microarray. Currently, we are using metagene analysis and other bioinformatic analysis methods we used previously to examine stage-specific changes in actively transcribed genes during murine GC development (Zhu et al 2016) using datasets from *Tg(Pcp2EGFoL10a)* TRAP mice. We are especially interested in whether reactomes for chromatin remodeling genes will change during PC differentiation. We will then be able to use metagene analysis to compare metagenes that change during murine PC development with datasets from hES-PC derived cells at different states of differentiation. To date, we have obtained microarray data from one set of hES-PCs cells that express the Pcp2 TRAP tag. Comparison of these datasets will allow an estimation of neuronal maturity as well as variations between mouse and human gene expression. These datasets will serve as a baseline from which TSC patient PCs can be compared.

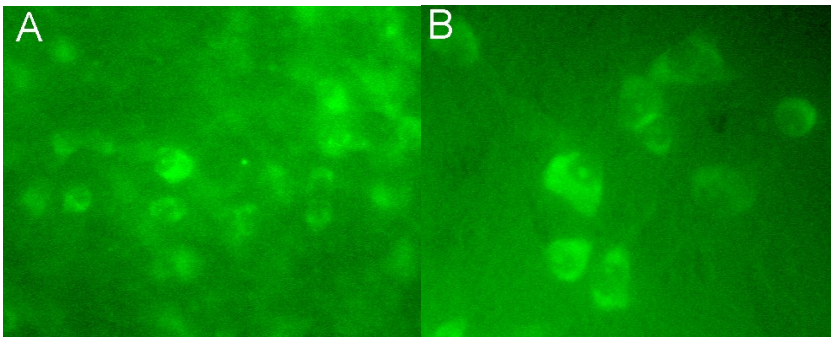

Figure 1. Live imaging of the Pcp2-EGFP-L10a TRAP-hESC line expressing GFP after 20 days of differentiation (A) and following isolation of hPSC-PCs on day 24 of differentiation followed by 24 days of co-culture with mouse granule cells (B). The EGFP-L10a fusion protein is localized to the nucleolus and perinuclear space as expected.

We at BCH have collected mRNA samples from control and TSC2 deficient patient derived hiPSC-derived premature PCs *in vitro,* and currently we are in the process of analyzing these transcriptional profiles in comparison to previous datasets of TSC2-deficient stem cell derived neural lineages. This data will provide important information of the effects of TSC2-mutations and mTORC1 overactivation in the early cerebellar lineage development from human stem cells.

For Aim 2. We at BCH have successfully generated CRISPR-cas9-mediated correction of TSC2-microdeletion in TSC-patient (*TSC2*+/-) derived hiPSC line (Figure 2). We used puromycin selection for isolation of the CRISPR-cas9 and ssODN transfected cells, and with this method we were able to isolate 5 individual hiPSC clones that contained the corrected TSC2-gene. The selected hiPSC-clones expressed pluripotency markers SSEA-4, OCT4, NANOG, TRA1-60 (Figure 2A). We also confirmed that the hiPSC clones had normal karyotype (Figure 2B). The gene correction was confirmed with insertion of silent mutation for DNA-cutting restriction enzyme to the CRISPR-cas9 transfected cells, followed by Sanger-sequencing of the correction site (Figure 2C).
We are now in a process of characterization of the cerebellar cell differentiation and PC differentiation capacity of the CRISPR-corrected hiPSC-clones in comparison to isogenic TSC2-mutant cell lines with heterozygous and homozygous microdeletion of the TSC2. We will study the synaptic marker expression and functionality of the cells to see if correction of the TSC2-mutation will rescue the discovered disease

phenotypes in vitro.

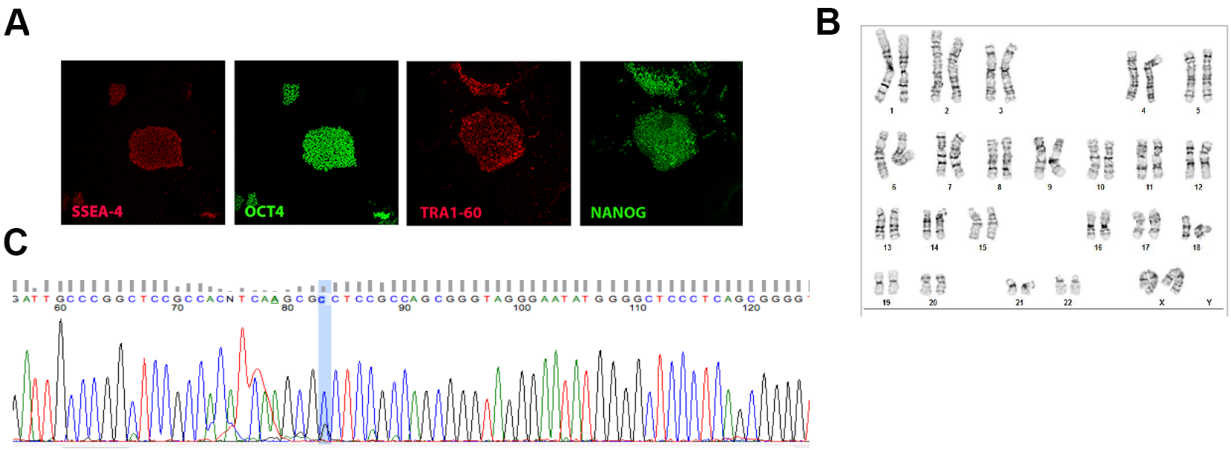

Figure 2. CRISPR-cas9 mediated TSC2-gene correction in *TSC2+/-* patient derived hiPSCs. A. Pluripotency marker expression SSEA-4, OCT4, TRA1-60, NANOG in CRISPR-cas9 and ssODN transfected and corrected hiPSC-clone. B. We detected the selected corrected hiPSC-clone had a normal karyotype. C. Sanger-sequencing was used to confirm presence of cloned restriction enzyme site in CRISPR-cas9 and ssODN tranfected and puromycin selected clone.

Work at Rockefeller is continuing to explore ways to speed up maturation of hPSC-PCs, with an extended dendritic arbor seen 79 days after isolation and co-culture with mouse granule cells (Figure 3).

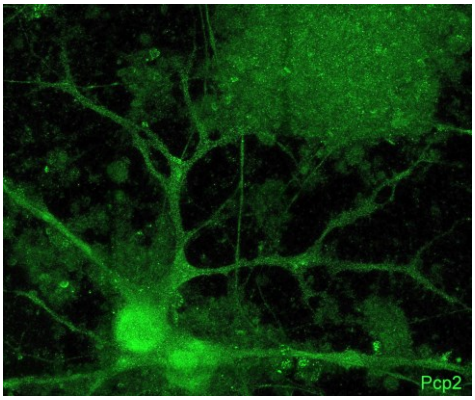

Figure 3. Confocal imaging of hPSC-PCs labeled with Pcp2 antibody following isolation of PCs on day 24 of differentiation followed by 79 days of co-culture with mouse granule cells shows an extended dendritic arbor typical of mature Purkinje cells.

For Aim 3. We at Rockefeller have begun developing methods for implanting hPSC-PCs into the neonatal mouse cerebellum. Preliminary results show good survival and proper targeting of hPSC-PCs into the cerebellum for up to 1 week post implantation however neurons failed to survive past this point. As implantation of human cells into a mouse may cause xenograft immune rejection, we are currently testing implantation into NOD/SCID immunodeficient mice.

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

During this period Dr Sundberg has participated in the annual ISSCR meeting, Boston 06/17/2017. This meeting covered areas of human stem cell differentiation, transplantation and quality control. It provided important information for the development more standardized differentiation protocols for pluripotent cells and quality control protocols.

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?

For Aim 1. Microarray datasets from differentiated hPSC-PCs will be compared to microarray datasets of mouse PCs at various ages. Pcp2-EGFP-L10a TRAP-TSC lines will be generated in order to compare patient PC gene expression to human and mouse control datasets.

For Aim 2. We are going to characterize the differentiation capacity of the CRISPR-cas9 corrected isogenic hiPSC-clones in parallel with isogenic patient hiPSC-line with heterozygous TSC2-microdeletion and isogenic hiPSC-line with bi-allelic TSC2-deletion. We will evaluate the gene-dosage effect of the TSC2-loss on the cerebellar precursor cell development and PC differentiation capacity, proliferation, dendritic and synaptic development and functionality *in vitro*. We will optimize multi-electrode array method for characterization of the functionality of the isogenic control line and TSC2-mutant hiPSC-derived PCs *in vitro*.

For Aim 3. We are testing implantation of hPSC-PCs into the neonatal cerebellum of NOD/SCID immunodeficient mice.

4. IMPACT:

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: The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

- Changes in approach and reasons for change.
- Actual or anticipated problems or delays and actions or plans to resolve them.
- Changes that have a significant impact on expenditures.
- Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Changes in approach and reasons for change
Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report.

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.

6. PRODUCTS:

• Publications, conference papers, and presentations

-Poster presentation in the ISSCR-meeting 06/14/2017, Boston:
Title: Characterization of TSC2 deficient human iPSC derived Purkinje cells in an in vitro model of autism.
Author list: Maria Sundberg, Ivan Tochitsky, David Buchholz, Kellen Winden, Deniz Cataltepe, Min-Joon Han, Clifford Woolf, Mary E. Hatten, Mustafa Sahin.

Journal publications.
Nothing to report.

Books or other non-periodical, one-time publications.
Nothing to report.

Other publications, conference papers, and presentations.

- **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

Provide the following information on participants:
- *what individuals have worked on the project?*
- *has there been a change in the other active support of the PD/PI(s) or senior/key personnel since the last reporting period?*
- *what other organizations have been involved as partners?*

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort).
• Provide the name and identify the role the person played in the project. Indicate the nearest whole person month (Calendar, Academic, Summer) that the individual worked on the project. Show the most senior role in which the person worked on the project for any significant length of time. For example, if an undergraduate student graduated, entered graduate school, and continued to work on the project, show that person as a graduate student, preferably explaining the change in involvement.
Describe how this person contributed to the project and with what funding support. If information is unchanged from a previous submission, provide the name only and indicate "no change".

This is a collaborative project between Boston Children's Hospital and Rockefeller University.

Name: Mustafa Sahin
Project Role: PI
Nearest person month worked: **1.2 cal months**
Contribution: As PI, Dr. Sahin has supervised all aspects of the research plan and coordinated the communication with Dr. Hatten's lab at Rockefeller University.

Name: Maria Sundberg
Project Role: postdoctoral fellow (BCH)
Nearest person month worked: **6 cal months**
Contribution: Dr Sundberg carried out progress reported in the Aim 2 on design of the CRISPR-cas9-mediated gene correction to TSC2-mutant hiPSC-line and neuronal differentiation and characterization of the corrected hiPSC-clone. She has also optimized functional characterization of these lines with multi-electrode platform and collected samples for transcriptional profiling of the maturing hiPSC-PCs *in vitro*.

Name: Mary Elizabeth Hatten
Project Role: Co-PI
Nearest person month worked: **1.2 cal months**
Contribution: Dr. Hatten supervised the work done on Aims 1,2 and 3 and coordinated the research plan with Dr. Sahin at Boston. She also traveled to Boston to meet with personnel there are review progress as well as to plan a publication that is currently in preparation.

Name: David Buchholz
Project Role: Subcontract Postdoctoral Fellow
Nearest person months worked: **6 cal months**
Contribution: Dr. Buchholz carried out the progress reported in Aims 1,2 and 3 on generating hES cell lines expressing a Pcp2 TRAP line, on further developing a protocol for differentiating Purkinje neurons and on developing a methodology to implant immature hES-derived PCs into mouse cerebellar cortex.

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

Name: Mustafa Sahin
Project Role: PI

New Active Support:

Early Biomarkers of Autism Spectrum Disorders in infants with Tuberous Sclerosis,
(Co-PI: Sahin & Krueger)
NIH U01 NS082320
National Institutes of Neurological Disorders and Stroke
6001 Executive Boulevard, Suite 3290, MSC 9537
Bethesda, MD 20892-9537
Contracting/Grants Officer: Vicky R Haines
Email: vhaines@mail.nih.gov Phone: 301-496-1365
09/01/2016 - 08/31/2018 – 1.8 calendar months
This is a multi-center clinical trial as a part of the ACE networks. The short term goal is to better characterize the autism phenotype of TSC and identify biomarkers that predict risk for development of autism in children with TSC.
Specific Aims:
Aim 1. To create an infrastructure for clinical treatment trials in TSC patients at risk for ASD
Aim 2. To characterize the developmental precursors of ASD in a large number of TSC infants using a prospective multi-center design **Aim 3.** To identify biomarkers with advanced DTI that help predict development of ASD in TSC infants **Aim 4.** To identify biomarkers with EEG that help predict development of ASD in TSC infants.
No overlap.

PACT (Preclinical Autism Consortium for Therapeutics) Proposal for Autism Speaks-Rugen Partnership
(PI: Sahin)
Autism Speaks/Rugen Therapeutics
106 State Road, 2nd Floor
Princeton, New Jersey 08540
Contracting/Grant Officer: Joan New (Grants Manager)
Ph: 609-228-7313 ; Email: jnew@autismspeaks.org
01/01/2017 - 12/31/2018 – 0.12 calendar months
The mission of the PACT is to discover pharmacological compounds with high efficacy in reversing autism-relevant behavioral phenotypes in the most robust rodent models.
No overlap.

A randomized double-blind placebo-controlled trial of Everolimus in children and adolescents with PTEN mutations (rare disease clinical research consortium-developmental synaptopathies consortium,
(PI: Sahin)
Charities Aid Foundation/PTEN Foundation
3rd Floor, Paternoster House; 65 St Paul's Churchyard; London EC4M 8AB
Contracting/Grant Officer: Siobhain McCullagh [mailto:siobhain@ptenresearch.org
03/01/2017 – 02/28/2018 – 0.60 calendar months
This proposal addresses a key area of interest: Pharmacological treatments of a well-defined subgroup with autism. Specifically, we propose a Phase I/II 6-month, randomized, double-blind placebo-controlled trial of everolimus with additional 6-month open-label extension in children and adolescents, ages 6 to 21 years with a PTEN mutation, with safety and neurocognition as the primary endpoints. The overarching purpose of this phase II pilot study will be to establish short-term safety of everolimus treatment in

individuals with germline PTEN mutations and evaluate associated cognitive and behavioral changes in this population.
No overlap.

Boston Intellectual and Developmental Disabilities Research Center U54HD090255
(PI: Pomeroy)
Eunice kennedy shriver national institute of child health & human Development
Program Official: Melissa Parisi
Email: parisima@mail.nih.gov Phone: (301) 435-6880 Fax: (301) 496-3791
Clinical/Translational core (Role: Director) calendar months Administrative core (Role: Associate Director) Annual Direct $0 0.12 calendar months
09/23/2016 – 05/31/2021
Dr. Sahin devotes the aforementioned effort on the administrative core to the IDDRC Executive Committee in helping to direct the Center. Additional he devotes effort to running the clinical/translational core of this project. This funding in no way supports Dr. Sahin's research activities.
No overlap.

Mechanisms of Synapse Remodeling in TSC
(MPI: Sahin & Stevens)
Boston Children's Hospital
300 Longwood Avenue. Contracting/Grant Officer: Kamei Miller
Phone: Email: 617-919-2729; osp@childrens.harvard.edu
09/23/2016 – 05/31/2021 – 0.24 calendar months
Drs. Sahin and Stevens are multiple PIs on a project to examine the cell intrinsic and extrinsic mechanisms mediating synaptic modeling and may inform new therapeutic targets and biomarkers for TSC and related neurodevelopmental disorders.
No overlap.

Salivary miRNA Biomarkers in Neurodevelopmental Disorders
(PI: Sahin)
Motion Intelligence, Inc.
505 Irving Ave, Suite 2102
Syracuse, NY 13210
Contracting/Grant Officer: Richard Uhlig
Phone: 607-227-4400. Email: Richard.uhlig@motionintel.com
11/11/2016 – 7/1/2018 – 0.12 calendar months
This study is aimed at identifying a diagnostic salivary miRNA profile for four single gene neurodevelopmental disorders (MECP2, FMRP, TSC, and CDKL5).
No overlap.

RAD001 and mTOR Novartis Tool Compound 1 (NTC1) testing in Tsc2KC+ mice, (PI: Sahin)
Novartis Institutes of Biomedical Research
250 Massachusetts Avenue
Cambridge, MA 02139
Contracting/Grant Officer: Marjorie Eiref
Email: Marjorie.eiref@novartis.com
11/02/2016 – 09/30/2018 – 0.12 calendar months
This project will pilot neuroPk study to select appropriate dose of compounds in Tsc2KC +animals, evaluate effect of RAD001 and WB-26-VB80 on epileptiform EEG activity in male Tsc2KC+ hypomorphic mice, and harvest tissues for bio chemistry and final PK analysis.
No overlap.

Neuronal excitability in TSC
(PI: Sahin)
Lam Therapeutics Inc.
530 Old Whitfield Street, Guilford, CT 06437
Phone: Email: 203-458-7100 ; info@lamthera.com
Contracting/Grant Officer: Henri Lichenstein (President & CSO)
05/19/2016 – 12/19/2017 – 0.01 calendar months
The goal is to test the efficacy of genetic anti-seizures medications on human TSC neurons. **No overlap.**

Boston Children's Hospital Clinical Site for the National Autism Cohort, (PI: Lisa Prock)
Simons Foundation Autism Research Initiative
160 Fifth Avenue, 7th Floor, New York, NY 10010
Contracting/Grants Officer: Calissia R. Franklyn, Grants Associate,
Phone: 212-604-8056, Email: calvarezfranklyn@simonsfoundation.org
03/01/2016 – 02/28/2019 – 0.36 calendar months (Co-I)
Researchers, as well as clinicians, at Boston Children's Hospital (BCH), involved with individuals with autism UAutism Cohort (CSN/NAC). Recruitment will occur via BCH and also with the help of community partners. Under the Translational Neuroscience Center staff will oversee recruitment activities, which include interfacing with 1) existing research-ready populations; 2) current/future clinical populations; and 3) potential research participants through community relationships.
No overlap.
New Completed Project:

Preclinical Autism Consortium for Therapeutics, (Co-PI: Sahin, Crawley, Paylor)
Autism Speaks
106 State Road, 2nd Floor
Princeton, New Jersey 08540
Contracting/Grant Officer: Daniel G. Smith (Senior Director, Discovery Neuroscience)
08/01/2015- 9/30/2016 – 0.12 calendar months
Specific Aims:
Aim 1. Identification of robust, well-replicated phenotypes relevant to autism in rodent genetic models.
Aim 2. Replication of phenotypes in a second cohort, and addition of mutant lines as needed, to generate a strong platform of 4 lines of mice and rats with mutations in genes implicated in autism, that display robust, well-replicated behavioral and physiological traits relevant to the diagnostic and associated symptoms of autism.

A Randomized, Double-Blind, Placebo-Controlled, Dose-Ranging Study of the Safety and Pharmacokinetics of Oral NNZ-2566 in Pediatric Rett Syndrome,
(PI: Sahin)
Neuren Pharmaceuticals, LTD
697 Burke Road, Suite 501
Camberwell VIC 3124, Australia
Contracting/Grant Officer: Jon Pilcher
Email: jpilcher@neurenpharma.com
03/01/2016 – 07/31/2017 – 0.12 calendar months
$111,013 Annual Direct

A randomized, double-blind, placebo-controlled dose ranging study of the safety and tolerability of NNZ-2566, also known as trofinetide, in female children and adolescents with Rett syndrome. This study will also investigate measures of efficacy and the pharmacokinetics of NNZ-2566 during treatment.

mTOR and protein synthesis in SMA
(Sahin, M. PI)
Cure Spinal Muscular Atrophy / Families of Spinal Muscular Atrophy
Contracting/Grant Officer: Kenneth Hobby (President)
925 Busse Road, Elk Grove Village, IL 60007
Phone/ Email: 800.886.1762 or email info@curesma.org
1/15/15 – 1/14/17 0.12 calendar
Annual direct:
The overall goal of this study is to characterize further the regulation of mTOR complex components and protein synthesis by SMN loss; and to explore rescue strategies for SMN-deficiency using mTOR activation.

A Phase 2b placebo-controlled cross-over study of the rh-IGF1 (mecasermin [DNA] injection) for treatment of Rett syndrome and development of Rett-specific novel biomarkers of cortical and autonomic function
(Sahin, M. PI)
International Rett Syndrome Foundation
4600 Devitt Drive, Cincinnati, Ohio 45246; jascano@rettsyndrome.org
Phone/Email: 513-874-3020
Grant/Contract Person: Janice Ascano, Ph.D. (Manager of Grants and Research)
8/01/12 – 12/31/16 0.36 calendar
Annual direct
This source is providing the main financial support for a randomized, double-blind, placebo-controlled, two-arm crossover study of IGF-1 in 30 children with Rett syndrome. We will collect extensive data on both efficacy and safety. The primary outcome measure is improvement in autonomic and respiratory function.

Project 2: Innovative Approaches to gauge Progression of Sturge-Weber Syndrome
(PI: Michael T. Lowton, MD)
NIH/NINDS 5U54NS065705 (Sub from The Regents of the University of California)
Sonny M. Carpio, Subcontract Officer, RMS
3333 California Street, Suite 315, San Francisco, California 94143
Phone/ Email: (415) 476-3109, feliciano.carpio@ucsf.edu
8/1/16 – 7/31/17 - 0.12 Cal Months (Role: Clinical Site Director)
The goal: Drs. Anna Pinto and Mustafa Sahin to enroll 5 subjects into the longitudinal study (Aim 1) and collaborate with the BVMC SWS network in this research. This funding is for gathering clinical data, urine samples, the NeuroQol data, photographs and filling out birthmark scores for these 5 subjects per protocol

Name: Mary E. Hatten
Project Role: Co-PI
New Active Support:

Starr Tri-Institutional Stem Cell Initiative Hatten (PI) 07/01/2016- 6/30/2019
Funding Agency: Starr Foundation
Title: Role of Tet and Chromatin Remodeling Genes in Human Cerebellar Neuron Synapse Formation and Function.

The major goal of this project is to study the effects of genetic perturbation of chromatin-modifying factors on the differentiation and synaptic physiology of hESC-derived cerebellar neurons. We will use CRISPR/Cas9 constructs to remove the Tet and other chromatin remodeling genes in hESC-derived GCs and PCs. Subsequently we will express these constructs in hESC-GCs or PCs and assay whether targeting Tet or chromatin remodeling genes affects cerebellar synapse formation when the human cells are co-cultured with mouse target. For targeted genes that decrease synapse formation by 50%, Hatten will use TRAP methodology to assay changes in gene expression, focusing on changes in axon guidance (dendrite formation) and ion channel genes, genes that we previously showed are altered by activation of Tet1/3 (Xhu et al, 2016). We will also transplant relevant differentiated hESC clones into mouse cerebellum to test their ability to integrate and form dendrites. Finally, Ryan will use a suite of biophysical approaches to examine the functional properties of synapses formed between hESC-derived cerebellar neurons and defined postsynaptic targets in vitro.
Role: PI (Co-PIs, Joseph G. Gleeson, M.D.; Timothy A. Ryan, Ph.D.)

Completed Support:
5R01NS051778-09 Hatten (PI) 05/01/2011-02/29/2016
Funding Agency: NIH/NINDS

Title: Role of Cdc42 and Par6 Polarity Complex in CNS Neuronal Migration
The goal of this research is to examine the role of the mPar6α polarity complex in the migration of CNS neurons along glial fibers during cerebellar development. The specific aims of this research are to investigate the other components of the mPar6α complex, the atypical PKCζ and Par3, to use genetic methods for chromophore-assisted inactivation of mPar6α in granule neurons and to examine upstream and downstream signaling pathways for the mPar6α complex in CNS glial-guided migration.
Role: PI

What other organizations were involved as partners?

Rockefeller University (as above, no change).

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- Financial support;
- In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);
- Facilities (e.g., project staff use the partner's facilities for project activities);
- Collaboration (e.g., partner's staff work with project staff on the project);
- Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each
- other's site); and
- Other.

8. SPECIAL REPORTING REQUIREMENTS:
None

9. APPENDICES:

Abstract of the poster presentation in the ISSCR-conference, 06/14/17, Boston:
Title: Characterization of TSC2 deficient human iPSC derived Purkinje cells in an in vitro model of autism.
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Abstract:
Tuberous sclerosis complex (TSC) is a neurodevelopmental disorder causing cortical tuber formation, epilepsy and autism. TSC1/2 mutations dysregulate the mechanistic target of rapamycin (mTOR) pathway and disturb neuronal protein synthesis, which leads to the development of ASD approximately in 50 % of all TSC-patients. Previous PET imaging studies have indicated that cerebellar deficits, such as cerebellar lesions and glucose hypermetabolism in deep cerebellar nuclei, are associated with a higher prevalence of autism in TSC-patients. However, the exact cellular deficits that occur during development of autism hasn't been investigated in detail with patient derived neural cells. We have previously created a mouse model lacking the Tsc1 gene specifically in cerebellar Purkinje cells (L7-Cre+;Tsc1f/f). This mouse model displayed autistic-like behavioral deficits including increased repetitive behaviors, decreased social interaction and cellular abnormalities (Tsai et al, 2012, Nature). Together, the clinical findings from TSC-patients and the phenotype of the Tsc1 conditional knock-out mice indicate that cerebellar Purkinje cells have a crucial role in the development of ASD. To characterize the disease phenotypes of TSC at the cellular and molecular level we have developed a novel differentiation protocol for generation of Purkinje cells from human iPSCs with patient specific TSC2-mutations. In this study, we derived pluripotent stem cell lines from three TSC-patients and four unaffected familial controls or age-matched controls for neuronal differentiation in vitro. We find that TSC2-deficient patient iPSC-derived PCs have mTOR-pathway hyperactivation that was detected with increased levels of phospho-S6 and phospho-S6 kinase. Our comprehensive RNA sequencing analyses revealed several interesting genes related to neuronal differentiation deficits of TSC2-mutant iPSC-derived Purkinje cells. We also detected deficits in the electrophysiological properties of hiPSC-derived PCs. According to our data, inhibition of mTOR-pathway may improve the electrophysiological properties of TSC2-deficient human Purkinje cells, and could be a target for pharmacotherapy for treatment of ASD in TSC.