PMS Distribution Study Descriptions

Contents

Study 115 – Exploring the Neuronal Phenotype of Autism Spectrum Disorders Using Induced Pluripotent Stem Cells

Summary

- **Grant Number:** R33MH087898
- **PIs:** Joachim F. Hallmayer, M.D., Ph.D., and Ricardo E. Dolmetsch, Ph.D.
- **Study Design & Aims:**
  - Autism spectrum disorders (ASD) are highly heritable complex neurodevelopmental disorders of the brain, which cannot be explained by mutation or mutations in any single gene. In the last couple of years, linkage and association studies have led to the identification of several mutations that confer susceptibility to ASDs. Studying the functional effects of these mutations offers a unique window to a better understanding of the underlying neurobiology.
  - One of the major obstacles is the difficulty in obtaining neurons and glial cells from patients with an ASD. The goal of this project is to develop the methods to convert skin cells from patients with ASDs into neurons and to characterize these neurons using high content screens. To achieve this goal we will convert fibroblasts into pluripotent progenitor (iPS) cells. In the next step we will differentiate these iPS cells into neurons in vitro. Finally we will study the specific cell- intrinsic aspects of neuronal function that are likely to be disrupted in ASDs including synapse formation, axonal and dendritic morphology and calcium signaling.
  - We have already established all of these techniques in our laboratory. Before we can apply these techniques on a larger scale we need to first address some of their limitations. The focus of R21 phase of the proposal is to improve and standardize the methodology. We will first generate and characterize iPS cells from human fibroblasts harvested from healthy controls and ASD patients with mutations in the CACNA1C and SHANK3 gene, mutations known to affect neuronal development, and optimize and characterize the differentiation of iPS cells (Specific Aim 1). We will develop standardized protocols for differentiating iPS cells into mixed populations of cortical, dopaminergic, and inhibitory neurons (Specific Aim 2). We will then characterize the cellular phenotypes of neurons from ASD and from controls, focusing on calcium signaling, dendritic arborization, and cell survival (Specific Aim 3). In the R33 phase of the project we will target a larger number of individuals with ASD a known to have a mutation in others gene/s affecting neuronal development (Specific Aim 4 and 5).

Acknowledgement

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R33MH087898 were Dr. Joachim Hallmayer and Dr. Ricardo Dolmetsch. Additionally, Dr. Hallmayer received supplementary funding for NIH grant R33MH087898 to obtain biomaterials, diagnostic assessments and other data from subjects with Phelan-McDermid Syndrome Foundation and their families. Dr Hallmayer supervised the diagnostic data collection on all projects. The collection of data and biomaterials would not have been possible without the generous help of the Phelan-McDermid Syndrome Foundation. Staff of the foundation offered invaluable assistance, support and guidance. Deepest gratitude is also due to the families and individuals with Phelan-McDermid Syndrome who were our partners in this research.

Publications

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- Yazawa M, Hsueh B, Jia X, Pasca AM, Bernstein JA, Hallmayer J, Dolmetsch RE. Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome. Nature 2011 Mar 10; 471(7337):230-234. PMCID: PMC3077925

iPSCs Generated by Secondary User

- Ilyas Singec generated new iPSC lines from fibroblast cell lines, which in turn were derived from skin punches obtained from 22 Phelan-McDermid syndrome and 13 controls from family members. All fibroblast cell lines were already part of the NIMH Repository study 115 and managed by Rutgers University/RUCDR Infinite Biologics. IBX/Sampled reprogrammed the fibroblasts to iPSC lines under contract. Singec's laboratory's immediate use of the iPSC lines was for projects related to the HEAL Initiative. The NCATS Stem Cell Translation Laboratory (SCTL) has developed a

scalable and fully automated protocol to generate pure cultures of sensory neurons (nociceptors) in large quantities under chemically defined conditions. The available screening capabilities, scalable production of the most relevant human cell types, and their real-time functional characterization provide unprecedented opportunities to, in collaboration with external pain/addiction experts, to identify probe/lead compounds with improved predictivity for in vivo human effects. In addition, Disease-in-a-dish iPSC-derived models utilizing iPSCs from patients with pain or addictive disorders may advance understanding of different types of pain, and differences in individual pain responses or risk of developing chronic pain or addiction upon opioid exposure.