Cerebral Organoids as a New Way to Model ADHD Pathophysiology: A Look at Molecular, Cellular and Connectivity Deficits

C. A. SARAIVA LOPES[1], S. KO[2], JANG. Y[1], KIM Y[1], J. R. COPPETA[3], B. M. COHEN[2], M.TEICHER[2], K.-S. KIM[2]
1-Dept. of Psychiatry, Mol. Neurobio. Lab., Belmont, MA; 2-Dept. of Psychiatry,
McLean Hosp., Belmont, MA; 3-Draper Labs., Cambridge, MA

#14578

McLean HOSPITAL
HARVARD MEDICAL SCHOOL AFFILIATE

HARVARD MEDICAL SCHOOL

ABSTRACT

The lack of a clear etiology of ADHD coupled with difficulties in recapitulating human brain development is limiting our understanding of its pathophysiology. While much remains to be clarified, it is now accepted that ADHD patients have altered brains.

The Prefrontal Cortex (PFC) has risen to be of central relevance to the neuronal pathways of ADHD.

Particularly, we propose that the root cause of the PFC's smaller structure involves a limited progenitor pool and impaired radial migration. To achieve these long-term goals, we used a novel episomal reprogramming method to generate high quality control ADHD-iPSC's and optimize in vitro organoid generation.

Our approach will facilitate examination of how disease risk is translated at the cellular and tissue levels through comparative studies of processes such as progenitor cell proliferation, migration and connectivity during development.

INTRODUCTION

-Attention deficit hyperactivity disorder (ADHD) is a common neurodevelopmental disorder, affecting between 5-10% of school-aged children.
-ADHD is highly heritable and multifactorial, and its core symptomatology includes inattention, hyperactivity/impulsivity, and motivational/emotional deficits.
-Mounting evidence characterizes the disorder with a reduced brain volume and delay in cortical maturation and its connecting brain regions. In addition, cortical thinning and folding are key pathophysiological features as well.
-While the molecular mechanisms underlying ADHD pathogenesis are unknown, recent studies place the prefrontal cortex (PFC) at the center stage of the neuronal pathway of ADHD, making it critical to understand the molecular influences that modulate PFC function in order to develop novel ADHD therapeutics.
-ADHD's pathophysiology appears to be rooted in embryonic development, emphasizing the need for a better model to study ADHD. Thus, prompting us to hypothesize that cerebral organoids derived from ADHD patients' induced pluripotent stem cells (iPSCs) can be developed as a platform to study ADHD's pathophysiology and to investigate the molecular and cellular phenotypes as an early ADHD brain surrogate system.

REFERENCES

-1.Barnett R (2016). Attention deficit hyperactivity disorder. Lancet. 387(10020): 737.
-2. Hansen, D.V., J.H. Lui, P.R. Parker, A.R. Kriegstein. (2010) Neurogenic radial glia in the outer subventricular zone of human neocortex. Nature, 464, pp. 554–561
-3. Lancaster MA, et al. Cerebral organoids model human brain development and microcephaly. Nature. 2013;501:373–379
-4.Monzel et al. A novel approach to derive human midbrain-specific organoids from neuroepithelial stem cells. Stem Cell Reports, 8, pp 1144-1154.

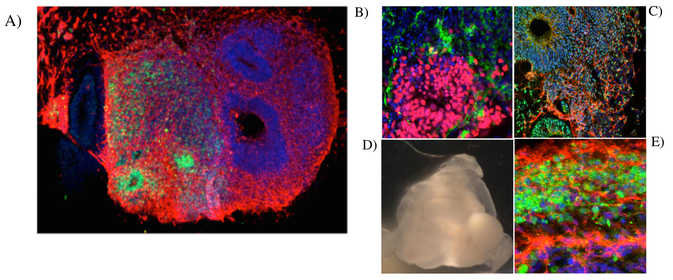
ACKOWLEDGEMENTS

. NARSAD Award for Young Investigator 2017
. Draper Laboratories

METHODS

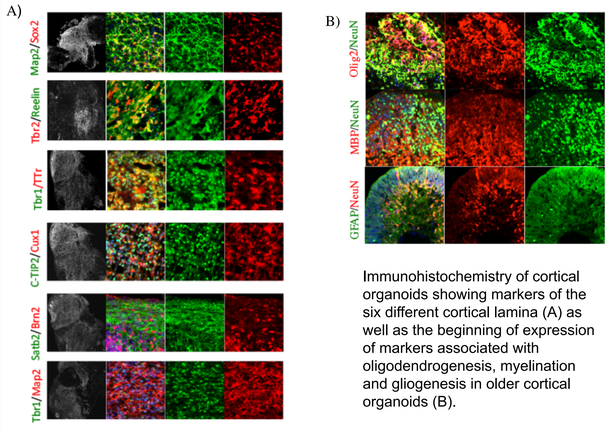
Generation of iPS-like clones from ADHD patients' fibroblasts:
-ADHD subjects recruited for biopsy fulfilled DSM-IV criteria, had no comorbid disorders and manifested objective symptoms of hyperactivity and inattention.
-We first established fibroblast cultures from four ADHD (EB, A09,IO2,A12) and four healthy (RB,A10,IO1,AMS) subjects following clinical evaluation and informed consent. Approximately 1 x 10^5 fibroblasts from each subject were electroporated with episomal plasmids. For three days after electroporation, cells were maintained in fibroblast media and supplemented with human PSC media for 4 days. From day 7 to day 15, cells were cultured with human iPSC media. 10 ng/ml basic fibroblast growth factor (bFGF) was added as supplement. We picked up granulated colonies with hES-like morphologies following approximately three weeks of culture. These iPS lines were established based on morphological criteria, stable expansion (at least 28 passages or more), ES marker expression (e.g., AP, SSEAs, Oct4, and Nanog;), and no chromosomal integration was confirmed by genomic PCR of EBNA1 gene.
Organoid generation:
-Cerebral Organoids were generated with 9,000 cells, according to the protocol from Lancaster et al.
Evaluation of electrophysiological activity
-Calcium imaging and multielectrode array (MEA/AXION) recording was used to analyze the spontaneous activity of organoids according to Monzel et al.

Cortical Gyrencephalic Organoids exhibit stereotyped organized progenitor behavior and maturation



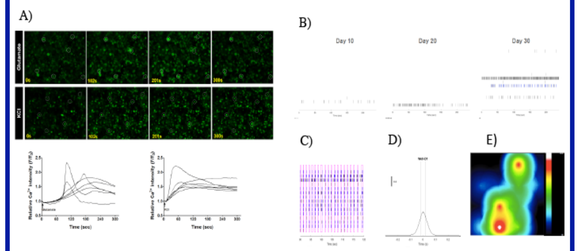
Immunocytochemistry shows protein expression of pluripotency markers from all eight generated iPSC lines (A). mRNA expression of pluripotency markers shows several fold increase of pluripotency markers in generated iPSC's comparatively to fibroblasts (B). PCR shows no chromosomal integration of reprogramming episomal plasmids in all iPSC lines generated for this study (C).

Cell diversity in cortical organoids



Immunohistochemistry of cortical organoids showing markers of the six different cortical lamina (A) as well as the beginning of expression of markers associated with oligodendrogenesis, myelination and gliogenesis in older cortical organoids (B).

Physiological maturation of cortical organoids



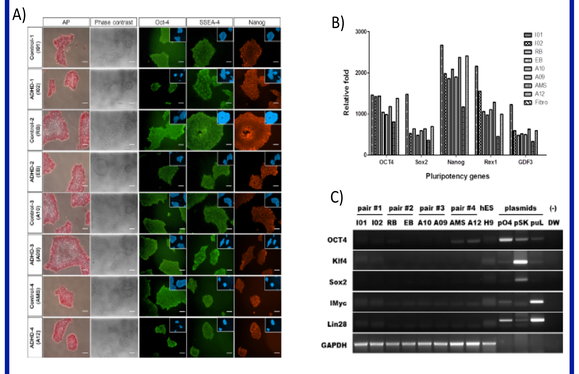
Cortical organoids exhibit neural activity. Exogenous glutamate application evokes an increase in calcium spike, indicating glutamatergic receptor activity in control derived organoids (A). Control derived cortical organoids exhibit increased number of spikes (B) as cultures mature as well as a network and synchrony pattern (C&D). Network synchrony is represented in the pink lines (C). Neuronal activity on a MEA plate at d30 of culture (D).

ADHD and Control Subjects Recruitment

Pair	Type of Subject	Date of Biopsy	Subject #	Comorbid Disorders	Gender	DOB	Samples Collected	Fibroblast?	iPSCs
#1	Control	2010-06-10	IPS01	No	F	1980-12-07	Skin	Yes	8 lines (6 vials/ea P-4 & P-5)
	ADHD	2010-06-10	IPS02	No	F	1979-01-03	Skin	Yes	3 lines (6 vials/ea P-4 & P-5)
#2	Control	2010-06-14	IPS03RB	No	M	1989-02-15	Skin	Yes	8 lines (6 vials/ea P-4 & P-5)
	ADHD	2010-06-23	IPS04EB	No	M	1992-04-27	Skin	Yes	6 lines (6 vials/ea P-4 & P-5)
#3	ADHD	2014-11-04	IPS09	No	M	1990-10-08	skin,blood	Yes	6 lines (6 vials/ea P-4 & P-5)
	Control	2014-11-04	IPS10	No	F	1995-07-01	skin,blood	Yes	6 lines (6 vials/ea P-4 & P-5)
#4	ADHD	2015-05-11	IPS_ADHD_12 (35369f)	No	F	1995-07-26	skin,blood	Yes	6 lines (6 vials/ea P-4 & P-5)
	Control	2015-06-09	IPS ADHD_MS (35435f)	No	F	1996-05-28	skin	Yes	6 lines (6 vials/ea P-4 & P-5)

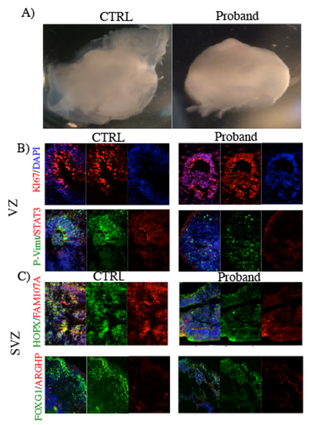
Four Caucasian ADHD subjects with no comorbid disorders were selected for the generation of multiple iPS-like clone candidates as well as four psychiatrically healthy gender matched siblings to be used as healthy controls.

Generation of ADHD iPSC's with episomal reprogramming method



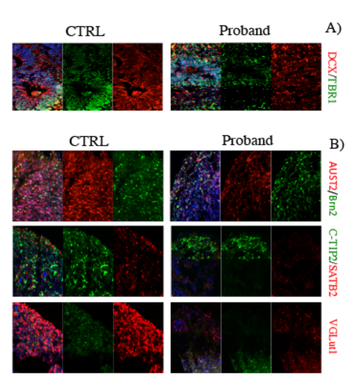
Immunocytochemistry shows protein expression of pluripotency markers from all eight generated iPSC lines (A). Several fold increase in pluripotency markers mRNA's comparatively to fibroblasts (B). PCR shows no chromosomal integration of reprogramming episomal plasmids in all iPSC lines generated for this study (C).

Generation and characterization of iPSC-derived cortical organoids from ADHD patients and their healthy siblings (controls); progenitor behavior



Whole view of forebrain organoids shows a partly reduced size of ADHD-derived forebrain organoids comparatively to controls at d25 (A). Control tissues display abundantly large neuroepithelial tissues (Ki67+) composed of progenitors cells; patient derived organoids display only occasional neuroepithelial regions with decreased thickness of vZ and RG's markers as given by p-Vimentin (B). ADHD derived organoids show a reduction in HOPX+ and FAM107A+ expression (C) comparatively to controls. FOXG1 is a hallmark forebrain marker and ARHGAP11B has been claimed to contribute to cortical folding and gyrification, which are attributes synonymous to intellectual capability. Remarkably, here we see a reduction in both factors, which could explain the somewhat smaller size of ADHD cortical organoids (C).

Generation and characterization of iPSC-derived cortical organoids from ADHD patients and their healthy siblings (controls); neuronal behavior



TBR1 is involved in differentiation and neuronal migration as well as normal brain development. Its expression is reduced in ADHD derived cortical organoids (A). AUST2, a marker of prefrontal cortex and a candidate for numerous neurological disorders, is impaired. Brn2, a marker of lamina II/III, is unchanged (B). cTip2 and SATB2, markers of lower and upper cortical laminae, respectively, are reduced, as is the glutamatergic transporter.

Conclusion

- Cerebral organoids display stereotypical organization and progenitor behavior: distinct corticogenesis markers with a clear pre-plate, cortical plate and the appearance of late markers associated with oligodendrogenesis; myelination and gliogenesis.
- Cortical organoids exhibit electrophysiological activity both by Fluo-4AM based Calcium imaging and the MEA system.
- We have generated ADHD forebrain derived organoids from one patient iPSC line and its matching control.
- In this pair, we saw that patient-derived forebrain organoids display reduction in size; reduction in outer RGC's (HopX+), migratory neurons (TBR1+), forebrain markers (FOXG1+), and cortical markers: c-TIP2 and SATB2.
- We will confirm the cortical phenotype in the remaining ADHD lines as well as generate ADHD-derived midbrain organoids.