Thank YOU for joining us!

Retreat Organizing Committee:

Manny Casanova (USC-Greenville)
Chris Cowan (MUSC)
Perry Halushka (MUSC)
Jane Joseph (MUSC)
Dayan Ranwala (MUSC)
Jane Roberts (USC)
Rich Steet (GGC)
Jeff Twiss (USC)
Michael Watson (MUSC)

Sponsors:

- Office of the Provost, MUSC
- SC Commission on Higher Education
- Office of Research, USC
- Greenwood Genetics Center
- Neuroscience Institute, MUSC
- South Carolina Clinical and Translational Research Institute, MUSC (NIH - UL1 TR001450)
South Carolina Autism and Neurodevelopmental Disorders (SCAND) Consortium – 2nd Annual retreat

Mission: to drive research excellence in South Carolina for clinical, translational and mechanistic studies aimed at understanding and treating neurodevelopmental and autism spectrum disorders through a state-wide, multi-disciplinary research consortium.

South Carolina institutions represented today:
College of Charleston, The Citadel, Claflin Univ, Clemson Univ, Furman Univ, Greenwood Genetic Center, USC and USC-Greenville School of Medicine
From Bench to Bedside and Back Again:
integrated approach to studying the genetics of a
neurodevelopmental disorder

- At the Bench: MEF2 proteins regulate activity-dependent synapse development
  Christopher W. Cowan, PhD, MUSC

- At the Bedside: human genome sequencing links MEF2C to syndromic autism
  Steven Skinner, MD, Greenwood Genetics Center

- Back to the Bench: modeling MEF2C haploinsufficiency syndrome in mice
  Adam Harrington, PhD, MUSC

- Into the Future: Insights into brain pathology and potential therapeutic strategies
  Catherine Bridges, BS, MUSC

Question and Answer period
At the Bench: MEF2 Proteins Regulate Activity-Dependent Synapse Development

Christopher W. Cowan, PhD
Professor of Neuroscience
William E Murray SmartState Endowed Chair in Neuroscience
Key Processes in Brain Development

- Differentiation & migration
- Axon guidance
- Synapse formation & remodeling
A synapse can excite the next cell or inhibit it.
An imbalance of excitation vs. inhibition can alter brain function

Neurotypical behaviors

Autism behaviors, sensory sensitivity, seizures, etc.
Typical brain development involves the formation and elimination of synapses
MEF2 Transcription Factors: 
Activity-dependent regulators of gene expression

Olson, Greenberg, Lipton, Bonni, Pryves, Mao and many others
MEF2 Activity Reduces Excitatory Synapse Number

Flavell & Cowan et al, 2006, Science
MEF2 Transcription Factors: regulators of excitatory synapse elimination

Dr. Kim Huber
UT Southwestern

Flavell & Cowan et al., 2006, Science
Pulipparacharuvil et al., 2008, Neuron
Fragile X Syndrome

Trinucleotide repeat expansion in the FMR1 gene that causes transcriptional silencing

Leading genetic cause of Autism and ID

Sensory hypersensitivity, ADHD, social anxiety & epilepsy
Increased synapse # in Fragile X Syndrome

Irwin et al., 2001
Dolen et al., 2007
FMRP is Required for MEF2-induced Synapse Elimination

**Wild-type**

**Fmr1 KO**

**Fmr1 KO + FMRP**

**RESCUE**

Pfeiffer et al., 2010, Neuron
MEF2 and FMRP regulate activity-dependent synapse elimination

Pfeiffer et al., 2010, Neuron
Tsai et al., 2012, Cell
Smith et al., 2014, Neuron
Wilkerson et al., 2014, Cell Reports

MUSC.edu
MEF2 TFs link to multiple Neurodevelopmental Disorders

MEF2C Haploinsufficiency Syndrome
MEF2C Haploinsufficiency Syndrome

Deletions in 5q14.3

DNA binding

MEF2C protein

Paciorkowski et al., 2013, Neurogenetics
MEF2C is highly expressed in forebrain neurons

postnatal day 4

young adult

Mef2c ISH

Nissl

cortex

Mef2c ISH

Nissl

Allen Brain Atlas
Mef2c conditional knockout in the mouse forebrain

Mef2c mRNA
Emx1-Cre

MEF2C protein that can't bind to DNA

Harrington et al., 2016, eLife
Mef2c conditional knockout mice display numerous NDD-related behaviors

1. Reduced social interaction
2. Reduced ultrasonic vocalizations
   (social-related communication)
3. Increased repetitive motor movements
4. Motor hyperactivity
5. Severe deficits in learning and memory
6. Altered Anxiety-like behavior

Harrington et al., 2016, eLife
Loss of Mef2c in mouse cortical pyramidal neurons alters E/I synapses

Harrington et al., 2016, eLife
Identification of MEF2C-regulated Genes

Harrington et al., 2016, eLife
Summary

1. MEF2 and FMRP work together to regulate excitatory synapse elimination

2. Deletions and mutations in MEF2C are associated with an emerging neurodevelopmental disorder (MCHS)

3. Selective loss of MEF2C in mouse cortex dysregulates hundreds of neuronal genes, including risk genes for ASD.

4. ...and it produces behaviors reminiscent of ASD and NDD symptoms

5. MEF2C regulates E/I synapse balance in the developing cortex
At the bedside:
Human Genome Sequencing Links MEF2C to Syndromic Autism

Steven A. Skinner, MD
Hannah Warren, MS CGC
March 1, 2019
**MEF2C**

Originally identified as critical gene in 5q14.3 microdeletion syndrome in 2010. Pathogenic variants within the gene have since been described. *MEF2C* haploinsufficiency appears to impair central nervous system function.

Common clinical findings:

- Intellectual disability
- Autism spectrum disorder
- Epilepsy
- Absence of speech
- Limited walking abilities
- Hypotonia
- Stereotypic movements
- Minor brain malformations

Genotype-Phenotype Correlation

Variability noted in symptoms associated with different alterations impacting the *MEF2C* gene

- However, limited numbers overall

Haploinsufficiency generally associated with severe intellectual disability, epilepsy, stereotypic movements, and brain abnormalities
Missense variants and smaller deletions seem to be associated with a higher chance of independent walking, a lower risk of refractory seizures, and some limited speech
Duplications seem to be associated with a milder phenotype of mild cognitive impairment, speech disorder, and microcephaly

Patient 1

Presented at age 9y8m with global developmental delay

Medical history:
› History of grand mal seizures, well controlled with medication
› Abnormal brain MRI with areas of heterotopia in left lateral region

Developmental history:
› Ambulatory with toe walking
› Babbles with a few words, mostly nonverbal communication
› Sleeping difficulties requiring medication, containment at night

Behavioral abnormalities:
› Repetitive hand movements including flipping pages in a book, hand flapping, hand mouthing
› Some hyperventilation
› Truncal rocking
› Love of water
› High pain tolerance
Patient 1

Numerous genetic and biochemical tests that were normal

Whole exome sequencing pursued in 2014
  › In frame duplication identified in MEF2C gene
  › c.120_125dupCTATGA
  › Paternally inherited- clinically significant?

Follow-up family studies showed father to be mosaic
Patient 2

Presented at age 14m for developmental delay and seizures

Medical history:
› Seizures developed at 4m
› Normal brain MRI
› Low muscle tone
› Tremulous
› Two small indentations with hair on the midline neck

Developmental history:
› Not sitting unsupported at evaluation
› Some babbling
› No behavioral concerns
Patient 2

Chromosomal microarray identified a 5q14.3 microdeletion of 109 kb
- Includes exons 1 and 2 of MEF2C gene
- De novo
Patient 3

Presented at age 17m for developmental delay and seizures

Medical history:
› Seizures developed at 6m
› Abnormal MRI with asymmetric appearance of the hippocampi
› Low muscle tone
› Some twitching movements

Developmental history:
› Was not sitting unsupported at initial evaluation
› Some noises with no babbling
› Bruxism, hyperextending of legs
› Potentially some pauses in breathing
› Reduced eye contact
Patient 3

Chromosomal microarray identified a maternally inherited variant of uncertain significance
145-gene Epilepsy Panel identified a missense variant in *MEF2C*
- c.90G>T (p.K30N)
- Not previously reported
- *De novo*
Patient 4

Presented at age 16m for global developmental delay and 5q14.3 microdeletion

Medical history:
› Abnormal EEG showing seizure activity at 14m
› Microcephaly
› Low muscle tone
› Failure to thrive

Developmental history:
› Not sitting unsupported at evaluation
› Few single words
› Repetitive hand movements with hand wringing
Patient 4

Chromosomal microarray identified 5q14.3 microdeletion of 1264 kb

- Encompasses entire MEF2C gene
- Not maternally inherited, father yet to be tested
- Presumed de novo
The Faces of *MEF2C*
# Features of our cohort

<table>
<thead>
<tr>
<th>MEF2C alteration</th>
<th>c.120_125 deletion up</th>
<th>Exons 1-2 deletion</th>
<th>p.K30N</th>
<th>Full gene deletion</th>
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Future Plans

Developing parent questionnaire to expand clinical knowledge regarding alterations involving MEF2C
Back to the Bench: Modeling MEF2C Haploinsufficiency in Mice

Adam Harrington, PhD
Postdoctoral Scholar
Cowan lab: Dept of Neuroscience
Medical University of South Carolina
MEF2C Haploinsufficiency Syndrome (MCHS)

Simons SFARI Gene
Paciorkowski et al., 2013. *Neurogenetics*
Greenwoods Genetic Center – Personal Communication

*Mutations Identified by Greenwood Genetic Center*
MEF2 Transcription Factors are Evolutionarily Conserved.

Potthoff and Olson, 2007.
Expressing MCHS MEF2C mutations

A 293-T Cells (HEK)

MEF2C constructs

B

anti-HA:800

Empty GFP 2C WT 2C G27A 2C K30N 2C L38Q 2C I46T 2C D40-C41

MEF2C-HA

GFP

C

MEF2C Relative Expression

mMEF2C (α1,β,γ)

Harrington et al., in prep
MCHS Mutations in MEF2C Disrupt DNA Binding

Fluorescent Probe: MRE (MEF2 Response Element) (C/T)TA(A/T)kTA(G/A)
Label: IRdye700

EMSA

Shifted Band Intensity (a.u.)

-500
0
500
1000
1500
2000
2500

EMSA_Raw Signal-Empty background_Combined

Modified from Signosis
Harrington et al., in prep
So…

MCHS mutations disrupt DNA binding of MEF2C.

Next…

Generate a heterozygous mouse model that disrupts MEF2C DNA binding.
Using genetic tools to generate $Mef2c^{+/-}$ mice

Harrington et al., in prep
Hypothesis

Loss of one copy of *Mef2c* (*Mef2c*\(^{+/-}\)) in mice will result in behavioral changes relevant to MCHS.

- Social deficits
- Communication deficits
- Repetitive behavior/stereotypy
- Hyperactivity
- Intellectual disabilities
- High pain tolerance
- Limited walking abilities
- Seizures
- Sleeping problems
**MEF2C Haploinsufficiency Syndrome:**

**Human vs Mouse**

- Social deficits
- Communication deficits
- Repetitive behavior/stereotypy
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$Mef2c^{+/-}$ mice interact less with a novel social mouse.
MEF2C Haploinsufficiency Syndrome:
Human vs Mouse

ASD-core domains

- Social deficits
- Communication deficits
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Ultrasonic Vocalization (USV) Recordings – Mouse Communication

Mice emit ultrasonic vocalizations as a way to communicate.

Harrington et al. eLife 2016;5:e20059. DOI: 10.7554/eLife.20059
Social communication deficits in adult *Mef2c*\(^{+/-}\) mice.

Harrington et al., in prep
*Mef2c*⁺⁻ mice show reduced vocalizations during maternal separation.
**MEF2C Haploinsufficiency Syndrome:**

**Human vs Mouse**

- Social deficits
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**Mef2c**<sup>+</sup>/<sup>-</sup> mice show hyperactivity and repetitive behaviors.

Harrington et al., in prep
**MEF2C Haploinsufficiency Syndrome:**

**Human vs Mouse**

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Mef2c<sup>+/−</sup> mice show normal fear learning. (Pavlovian Fear Conditioning)

Training:

- Acclimate (2 mins)
- Tone (30 sec) 1 min
- Tone (30 sec) 1 min
- Tone (30 sec) 1 min

Tone: 80 dB; 30 sec
Shock: 0.5 mA; 2 sec

FC-Context Test (M+F)

FC-Cue Test (M+F)

Harrington et al., in prep
Mef2c+- mice do not show intellectual disabilities.

Other cognitive test:
Spatial learning (Barnes maze)
Working memory (Novel object recognition; y-maze)
Operant learning (Sucrose self-administration (SA))
Discrimination learning (Sucrose SA)
Extinction learning (Sucrose SA)

Harrington et al., in prep
**MEF2C Haploinsufficiency Syndrome:**

**Human vs Mouse**

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Mef2c<sup>+</sup>-/- mice have reduced responses to shock.

Harrington et al., in prep
**MEF2C Haploinsufficiency Syndrome:**

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*Mef2c*<sup>+/−</sup> mice have normal motor coordination.
**MEF2C Haploinsufficiency Syndrome: Human vs Mouse**

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Collaboration with Dr. Robby Green (UTSW)
Summary

*Mef2c*<sup>+</sup>-<sup>−</sup> mice display some behaviors similar to those reported in *MEF2C* Haploinsufficiency Syndrome (MCHS) individuals.

*Mef2c*<sup>+</sup>-<sup>−</sup> mice may be a relevant animal model for studying brain and behavioral dysfunction in MCHS and for testing candidate therapeutics.
Challenges

Heterogeneity of symptoms in MCHS individuals
  › Refining the phenotype of MCHS

*MEF2C* deletions vs point mutations

Genetic variation in humans
  › Polygenic risk alleles

Environmental influences

A mouse in NOT a human…