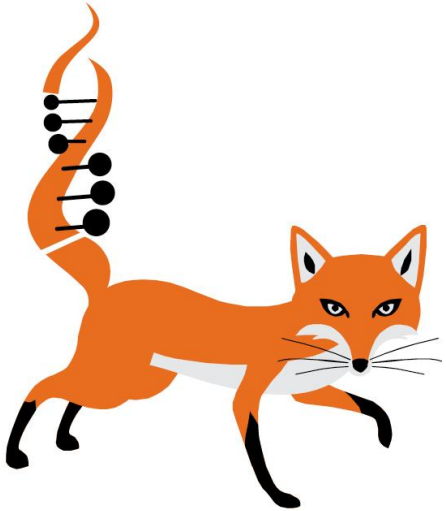


INTERNATIONAL
FOXG1
FOUNDATION®

The Amazing Gene That Could solve many brain-related disorders

Purpose of this presentation

The International FOXG1 Foundation (IFF) is providing grants to scientists to understand the biology of FOXG1, with the ultimate goal of identifying innovative therapeutic strategies.

This presentation seeks to inform scientists of current research on FOXG1 and assets available.

Link to Grant Application:
http://foxg1.org/wp-content/uploads/sites/17/2017/06/Funding-app.pdf

Please email application to nasha.fitter@foxg1.org

International FOXG1 Foundation (IFF)

IFF exists to pioneer innovative research to find a cure while guiding and supporting families.

- Formed on October 4, 2012 by six affected FOXG1 families
- Approved for 501(c)(3) nonprofit tax exempt status
- Raised $300,000 to date with a goal of $5 million by 2018
- FOXG1 Clinics at Boston Children's Hospital and Stanford's Lucile Packard Children's Hospital
- Ongoing Natural History Study with NIH Grant
- Active global parent community with 300 affected families

FOXG1 Foundation Scientific Advisory Board

Dr. Heather Olson, ***MD, MS, Children's Hospital Boston, Assistant in Neurology, Instructor of Neurology, Harvard Medical School.***

HARVARD MEDICAL SCHOOL

Dr. Orrin Devinsky, ***MD. NYU Langone Medical Center, Professor, Department of Neurology, Department of Neurosurgery, Department of Psychiatry, Chief of Service, NYU Epilepsy Service.***

NYU Langone MEDICAL CENTER

Dr. Walter Kaufmann, ***Ravenel Boykin Curry Chair in Genetic Therapeutics, Professor of Neurology, Greenwood Genetic Center.***

Greenwood Genetic Center

Elli Brimble, ***MSc, MS, CGC, Lucile Packard Children's Hospital at Stanford*** has joined us as the foundation's ***Genetics Liaison.***

Stanford MEDICINE

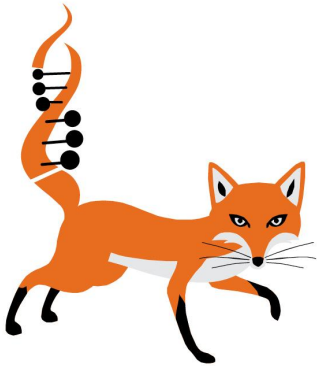
Research Goals - what we are looking to fund

1. **Understand the Biological Impact of FOXG1**
   Characterize the molecular, behavioural, and functional consequences of *FOXG1* disruption using patient-derived iPSC neurons and mouse models

2. **Utilize Gene Therapies for Cures**
   Exploit existing gene therapies to better understand *FOXG1* function, and to develop novel treatment strategies:
   - saRNA- and AAV-*Foxg1*-mediated approaches to boost endogenous expression
   - CRISPR/Cas9 editing as a tool to correct *FOXG1* mutations in neurons
   - RNAi as a tool to silence dysfunctional protein

3. **Target FOXG1 Symptoms**
   Promote transcriptomic and proteomic evaluations of FOXG1 to identify biological pathways that can be modulated with existing compounds

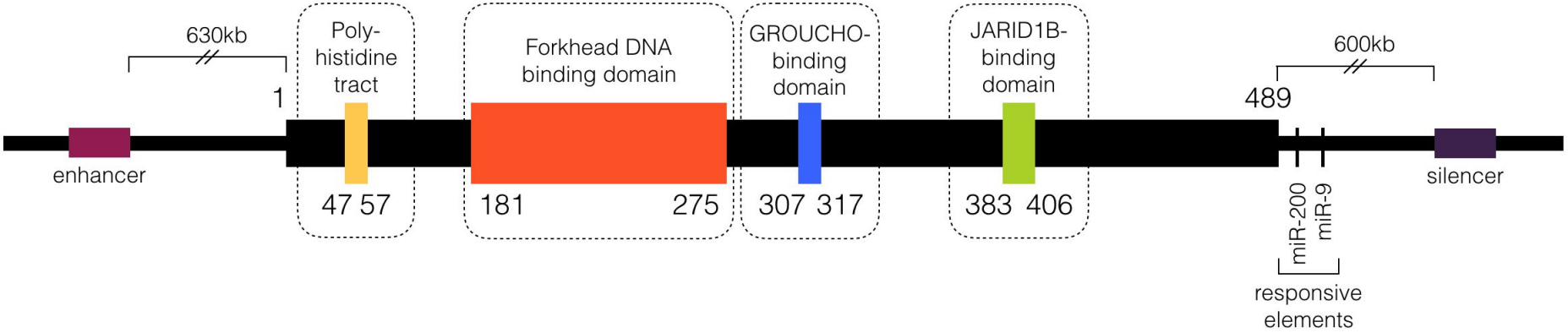
FOXG1 Science



FOXG1 is critical for brain development

- *FOXG1* encodes the Forkhead box G1 protein
- *FOXG1* is one of the **earliest transcription factors** that contributes to development of the telencephalon - the telencephalon is an embryonic structure that gives rise to the cerebrum
- The following pathogenic variants have been observed in *FOXG1* patients: deletions, duplications, missense, truncation and frameshift variants

Functional/regulatory elements in *FOXG1*



- All pathogenic missense mutations thought to occur in the forkhead DNA binding domain
- Deletions including *cis*-acting regulatory elements but not coding region result in a *FOXG1* syndrome phenotype

Established transcriptional targets of *FOXG1* (direct and indirect)

- FOXG1 thought to act primarily as a transcriptional repressor
- In addition to listed genes, widespread disruption of 'ventral' telencephalon genes with *Foxg1* deletion

Altered transcriptional profiles

Foxg1$^{+/-}$
Foxg1$^{-/-}$

↑ *Grid1*	↑ *Oxt*	↑ *Foxo1**	↑ *Foxo1*
↓ *Vglut1*	↑ *Avp*	↑ *Foxo3**	↑ *Bmp2*
↓ *Gria1*	↑ *Nnat*	↑ *Igf1**	↑ *Bmp4*
↓ *Gabra1*	↑ *Tgfb1*	↑ *Igf2**	↑ *Bmp6*
↓ *Dlg4*	↑ *Tgfb2*	↑ *Igfbp2**	↑ *Bmp7*

iPSC

↑ *GRID1*	↓ *GABRA1*		
↓ *VGLUT2*	↓ *DLG4*		
↓ *GRIA1*	*RELN***		
↓ *GRIN1*			
↑ *GAD1*			

*With TGFβ1

**FOXG1 shown to be enriched for in *RELN* promoter in NIH-3T3 cells, acting as a transcriptional repressor

Baek ST, *et al.* 2015. *Nat Med.* 21:1445-1454; Frullanti E, *et al.* 2016. *Eur J Hum Genet.* 24:252-257; Hanashima C, *et al.* 2002. *J Neurosci.* 22: 6526-6536; Manuel MN, *et al.* 2011. *Neural Dev.* 6:9; Patriarchi T, *et al.* 2016. *Eur J Hum Genet.* 24:871-880; Vezzli R, *et al.* 2016. *Oncotarget.* 7:37436-37455.

FOXG1 syndrome phenotypes

Microcephaly and Structural Brain Abnormalities

- Small head size with partial or full loss of the corpus callosum (inhibiting communication of left and right brain)

Epilepsy/Seizures

- Infantile spasms and life-threatening seizures resistant to drug and surgical treatments

Gross Motor/Fine Motor Delays

- Low muscle tone leads to inability to sit, walk, talk or use hands purposefully
- Strabismus, poor eye contact, and cortical visual impairment

Feeding Issues

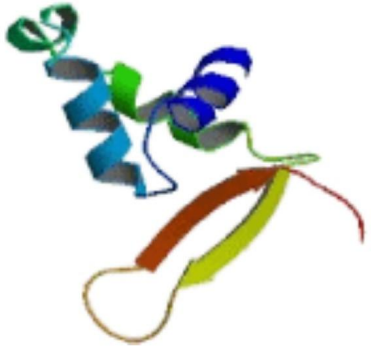
- Low muscle tone leads to reflux, constipation
- Many individuals require feeding tube placement

Associated Conditions & Disorders

- Autism Spectrum Disorders (ASD)
- Sensory Processing Disorder (SPD)
- Movement disorders

FOXG1 syndrome – history and landscape

- Formerly called Congenital Rett syndrome; has since been proven that Rett Syndrome is a sister to FOXG1 Syndrome, not a parent
- FOXG1 gene discovered in 1995 by Dr. Alessandra Renieri, University of Siena, Italy
- Shown as cause of Congenital Rett/FOXG1 Syndrome in 2005
- Currently 272 cases reported worldwide.
    - This number steadily increasing as more children undergo genetic testing for Autism Spectrum Disorder (ASD), epilepsy, etc.



FOXG1 Protein Representation

FOXG1 and brain development

Development of the Telencephalon:

- *FOXG1* is required for patterning the developing telencephalon, particularly for 'ventral identity' - 'ventral' denotes a region of the developing telencephalon that will become the basal ganglion

- The pattern of *FOXG1* expression in the developing telencephalon is a gradient - an abnormal 'ventral' region leads to corresponding defects in the opposite 'dorsal' region

- Mice engineered to lack *Foxg1* expression show gross malformations of the cerebral cortex, with a smaller, irregularly shaped cortex, and nearly absent ventral telencephalon

Neurogenesis and Cell Proliferation:

- *Foxg1* plays a role in the timing of neurogenesis and patterning of the cerebral cortex:
  - *Foxg1* suppresses early-neuronal cell types - allows for switch to neurons that reside in deeper layers of the cerebral cortex

- Several publications support a role for *Foxg1* in improving survival of neuronal progenitor cells (early cell types that give rise to neurons)
  - Some evidence to suggest that this does **NOT** require the DNA-binding activity of FOXG1
  - Possible avenue to explore genotype-phenotype correlations

Unlocking the mysteries of common neurodevelopmental disorders via *FOXG1*

Autism spectrum disorders: Proportion Monogenic - 15-34%

- *FOXG1* variant identified in an individual with ASD and his similarly affected mother
- *FOXG1* gene dose associated with ASD diagnosis

Epilepsy: Proportion Monogenic - >40% in epileptic encephalopathies

- ~87% of individuals with a *FOXG1* mutation are diagnosed with epilepsy
- Age of onset, seizure type, response to medication variable

Schizophrenia: Proportion Monogenic ??; heritability 70-80%

- A schizophrenia-associated loci was shown to physically interact with and regulate *FOXG1* expression
- Expression of schizophrenia-associated gene, *GRID1*, found to be significantly elevated in *FOXG1* patient-derived iPSC neurons and *Foxg1+/-* fetal mouse brain

Alvarez-Mora MI, *et al.* 2016. *Mutat Res Fund Mol Mech Mut.*784-785: 46-52; Finucane B & Myers SM. 2016. *Curr Genet Med Rep.* 4:147-153; Helbig KL, *et al.* 2016. *Genet Med.* 18:898-905; Patriarchi T, *et al.* 2016. *Eur J Hum Genet.* 24:871-880; Seltzer LE, *et al.* 2014. *Epilepsia.* 55:1292-1300; Won H, *et al.* 2016. *Nature.* 538:523-527.

A role for *FOXG1* in autism spectrum disorders?

Mariani J, *et al*. show…

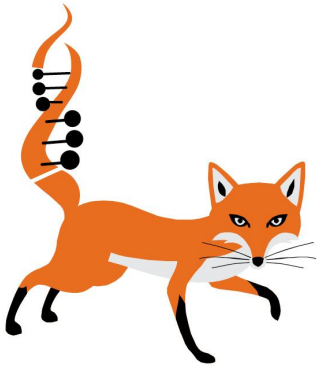
- iPSC neurons derived from patients with an autism spectrum disorder + macrocephaly (larger head size) show:
  - Overproduction of GABAergic progenitors and neurons (principle inhibitory neurons in adult brain)
  - Disrupted balance of inhibitory and excitatory neurons
  - Increased *FOXG1* expression

- Normalizing *FOXG1* expression using RNAi corrects for these findings, implicating *FOXG1* in ASD pathogenesis

- *FOXG1* expression significantly correlated with head circumference in this population, a marker of severity in ASDs (↑head circumference = ↑severity)

Mariana J, *et al.* 2015. *Cell.*162:375-390.

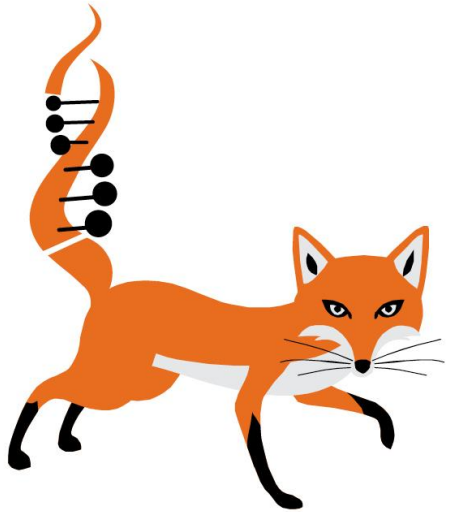
FOXG1 Assets for Scientists



FOXG1 promising for research investment

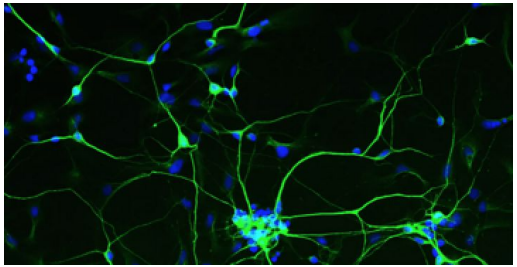
We know:

- Affected Gene
- Emerging genotype-phenotype correlation, with patients sharing strong similarities
- Strong research connecting FOXG1 to prevalent neuropsychiatric disorders
- How to safely deliver drug/gene therapies to the brain



Current assets for investigators

- Publicly available *Foxg1* deletion **mouse models** and **iPSC lines** for molecular/behavioural research and preclinical trials
- *FOXG1* clinics at Boston Children's Hospital and Stanford Children's Health to accumulate accurate clinical data
- Rett-associated Natural History Study to characterize outcomes across patients lifespan, and to explore possible genotype-phenotype correlations
- Active social network of affected families that can be reached immediately
- Development of a public-facing clinical and patient derived database

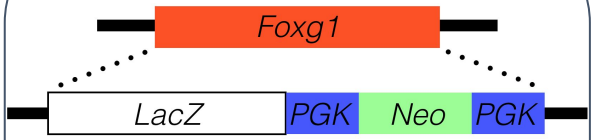


Boston Children's Hospital
Until every child is well℠

Stanford Children's Health

Existing *Foxg1* 'knock-out' models

***Foxg1-Lacz* Model**

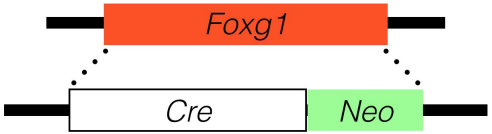


Foxg1 replaced by a LacZ + PGK/Neo cassette

- Homozygotes die shortly after birth
- Cerebral hemispheres reduced by 95% in homozygotes
- Almost all telencephalon expresses dorsal markers (no ventral structures)

Xuan S, *et al.* 1995

***Foxg1-Cre* Model**



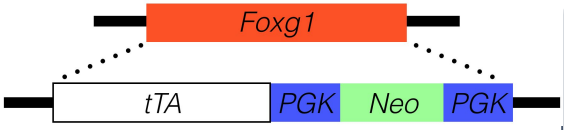
Foxg1 replaced by a Cre/Neo cassette

In heterozygotes:

- Significant reduction in volume of adult forebrain and thalamus
- Significant reduction in cortical thickness (could be due to *Cre* transgene)
- Expression analysis: ↑ oxytocin, ↑arginine vasopressin, ↑neuronatin

Hébert JM & McConnell SK. 2000

***Foxg1-tTA* Model**



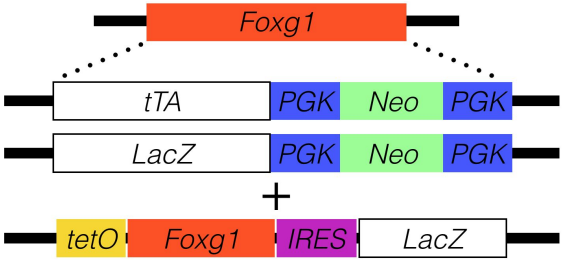
Foxg1 replaced by a tTA + PGK/Neo cassette

- Significant reduction in volume of adult forebrain in heterozygotes
- Heterozygotes show hyperactivity and impaired contextual fear conditioning

Hanashima C, *et al.* 2002

Other *Foxg1* Mouse Models

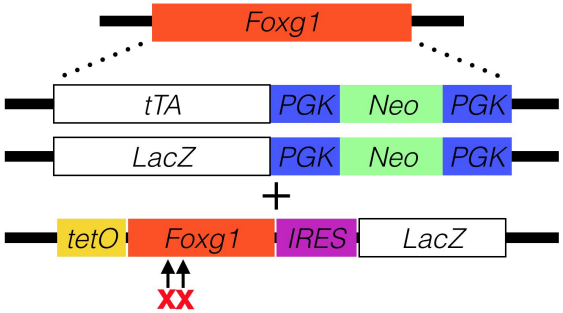
Conditional *Foxg1* Knockout



- Endogenous *Foxg1* expression absent; replaced with *Foxg1* transgene that can be 'turned off' with doxycycline treatment

Hanashima C, *et al.* 2007

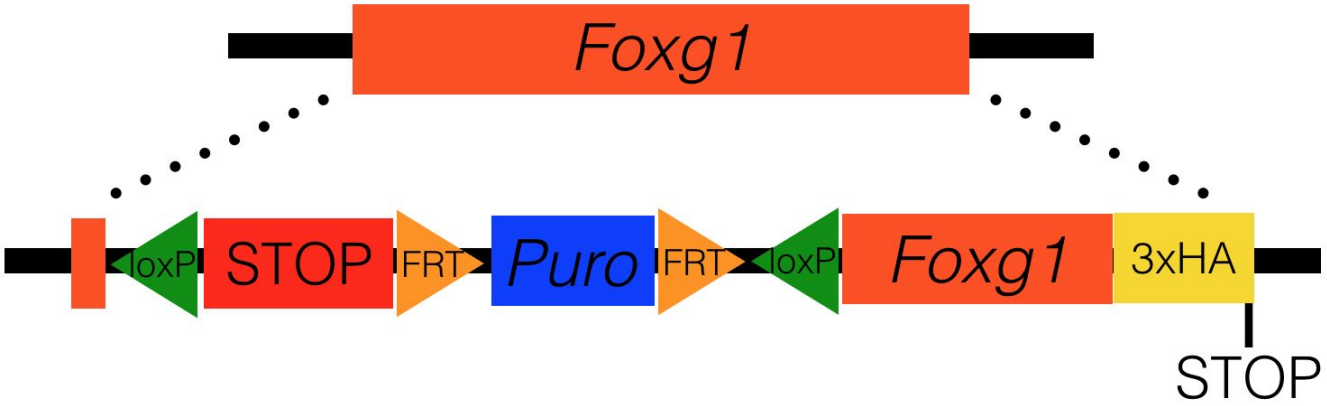
DNA-Binding Defective *Foxg1*



- Endogenous *Foxg1* expression absent; replaced with *Foxg1* transgene that cannot bind DNA
- Enhanced growth of telencephalon compared to knockouts

Hanashima C, *et al.* 2002

Newly developed Foundation-sponsored model



- Mouse model can generate:
  - Conditional knock-out (stop codon prevents *Foxg1* transcription)
  - Expression of 3xHA-tagged FOXG1 protein

iPSC lines available in Biobank

The following iPS lines are available for research purposes by Dr Renieri:

- Line ID 146/#14: p.Trp255*
- Line ID 611/#2013: p.Glu154Glyfs*301
- Line ID 968/#18: p.Ser323fs*325
- Line ID 2362/#5: hg19 chr14:g.28552714_29655318del (whole gene deletion)

Instructions to access lines:

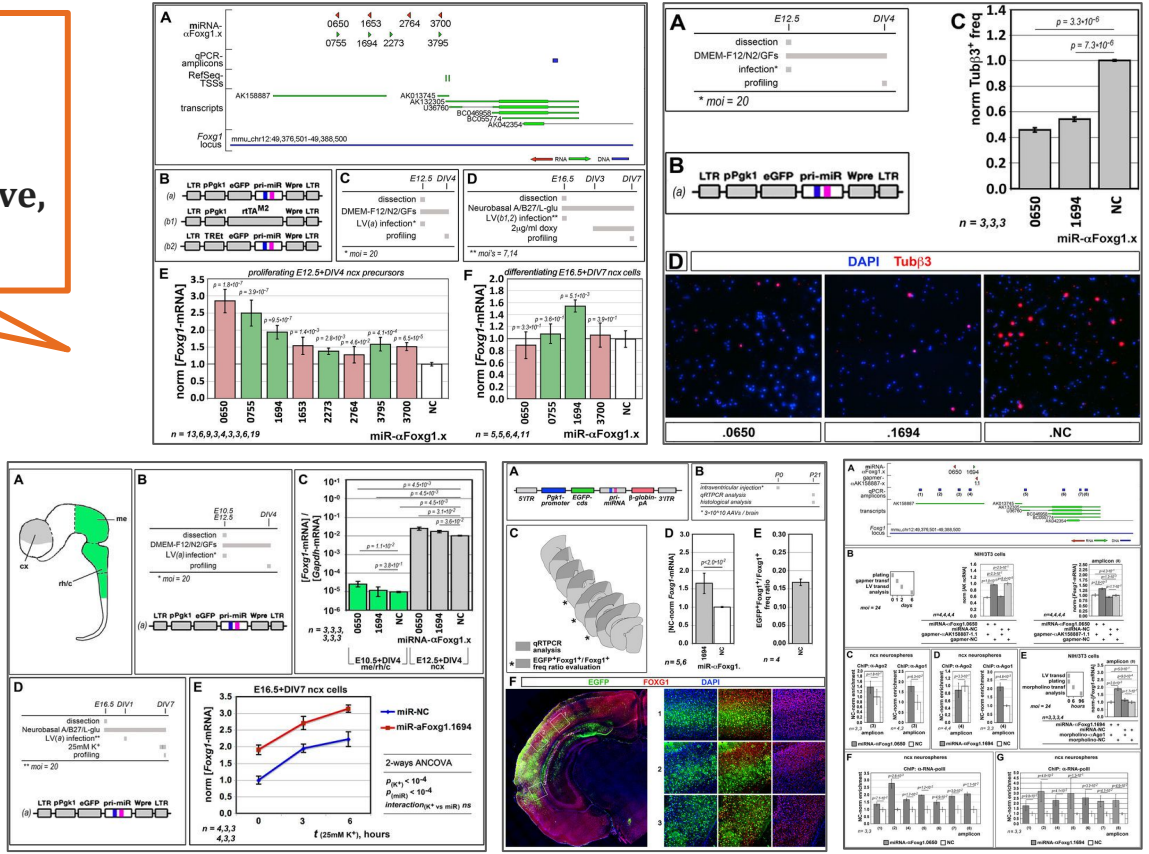
- Go to the web site http://biobanknetwork.telethon.it
- Select “login” option (on the lower left corner) and then “register”.
- Fill in the form fields (mandatory), and then confirm the registration. You will receive immediately username and password by email.
- Once you receive username and password you can log in and place your request:
- Select “Catalogue” and then “Request submission form”.
- You will be redirected to a page. Indicate title, granting agency and Grant number (if you have funded project, otherwise you can write “no grant”) and a brief description of the research project for which the sample/samples will be employed. At the end of the description you have to add the following sentence to specify the samples you need: “ I request FOXG1 iPSCs from patients with mutations in FOXG1 gene. The samples are present in the Biobank in Siena directed by Prof. Alessandra Renieri”.

Report on first FOXG1 gene therapy mouse trial

We chose to **administer miR-αFoxg1.1694** to the living brain through **AAV9**-pseudotyped, self-complementary **AAV2-derivative, adeno-associated viral vectors**

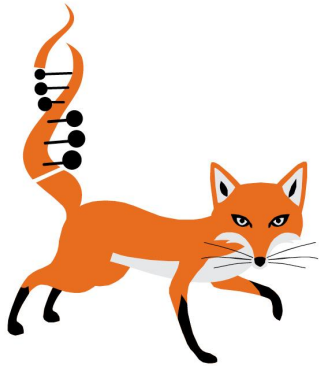
"**Remarkably, *Foxg1* was upregulated** by 1.66 ± 0.30 folds"

"Based on results reported above, **RNAa might be a simple and scalable approach for fixing this class of problems**"



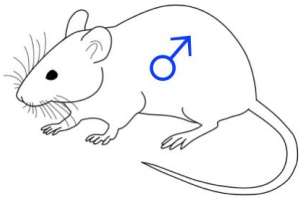
RNA activation of haploinsufficient *Foxg1*gene in murine neocortex, Cristina Fimiani, Elisa Goina, Qin Su, Guangping Gao & Antonello Mallamaci, *Scientific Reports* 6, Article number: 39311 (2016)

Lessons from other genetic neurodevelopmental disorders



Gene therapy stabilizes/improves symptoms in Rett syndrome mouse model

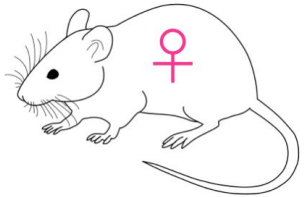
Mecp2 stop/y
4-8 weeks



\+
scAAV9/*cre*

- Improved survival
- Robust behavioural recovery

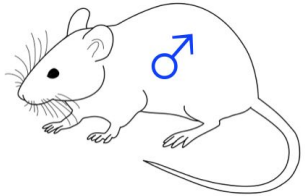
Mecp2 stop/+
7-10 months



\+
scAAV9/*cre*

- Symptom severity stabilized
- No seizures
- ↑ brain size and cognition

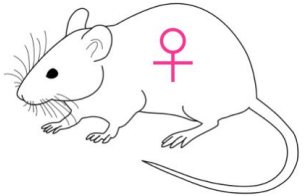
Mecp2 null/y
4-8 weeks



\+
scAAV9/*Mecp2*

- Physiologic expression levels
- Improved survival
- Phenotype stabilized

Mecp2 null/+
10-12 months



\+
scAAV9/*Mecp2*

- Symptom severity improved
- No seizures
- ↑ cognition

Garg SK, *et al.* 2013. *J Neurosci.* 33:13612-13620.

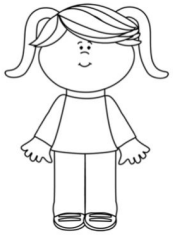
Human recombinant IGF-1 in Rett and Fragile X syndromes

IGF-1 impacts CNS via two pathways:

- PI3K-Akt-MTOR
- RAS-MAPK-ERK

AKT implicated in functional regulation of FOX transcription factors

MECP2 $^{x/+}$

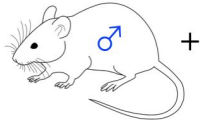


n=10
(20 wk)

\+ Mecasermin (rhIGF-1) →

- ↑ IGF-1 levels in CNS
- Acceptable safety and tolerability
- Improved apnea index
- Improved measures of anxiety
- No change in disruptive behaviours, communication, or motor indices

Fmr1 $^{-/y}$



(28 wk)

\+ NNZ-2566 (IGF-1, terminal tripeptide) →

- Corrected dendritic spine phenotype
- Improved hyperactivity and low anxiety behaviours
- Improved learning and long-term memory
- Improved socialization

Deacon RMJ, *et al.* 2015. *Neuromol Med.* 17:71-82; Kwaja OS, *et al.* 2014. *Proc Acad Natl Sci.* 111:4596-4601

Ongoing *FOXG1* research projects

Understand the Biological Impact of FOXG1

Boston Children's Hospital
Professor, Univ of California, San Diego

Develop patient-derived iPS neurons to characterize impact of FOXG1-disruption, and to provide a substrate for drug screening

Jeffrey Neul, MD, PhD
Professor, Univ of California, San Diego

Generate a *Foxg1*-deletion mouse model to characterize molecular, developmental, and behavioral outcomes of *Foxg1* disruption and subsequent correction

Alessandra Renieri, MD, PhD
Professor, Univ of Siena

Employ CRISPR/Cas9-mediated approach to correct *FOXG1* mutations in iPS neurons and animal models, and characterize outcomes

Flora Vaccarino, MD
Professor, Yale School of Medicine

iPS neurons to model neurodevelopmental disorders; *FOXG1* in the pathogenesis of autism spectrum disorders and head circumference

Antonello Mallamaci, PhD
Associate Professor, SISSA

Roberta Cilio, MD, PhD
Director of Pediatric Epilepsy Research, UCSF

Generate patient-derived iPS cortical neurons to model morphologic and electrophysiologic sequelae of *FOXG1* disruption, with special interest in cortical development

Utilize Gene Therapies for Cures

Guangping Gao, PhD
Professor, Univ of Mass. Medical School

Develop saRNA + AAV-mediated gene therapies for neurodevelopmental disorders, including *FOXG1*

Improve FOXG1 Symptoms

Sandra Acosta-Verdugo, PhD
Post-Doctoral Fellow, Northwestern University

Functional characterization of FOXG1 regulatory elements to identify possible therapeutic targets