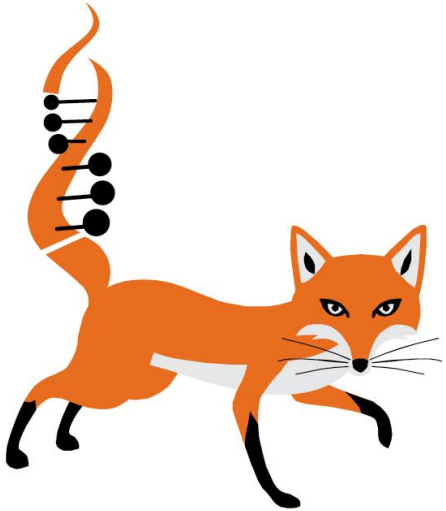


INTERNATIONAL
FOXG1
FOUNDATION®

**The Amazing Gene That Could** solve many brain-related disorders

# Purpose of this presentation

The International FOXG1 Foundation (IFF) is accepting proposals from 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/2017/10/Research-Proposal-Submission-Form.pdf

Please email application to adam.haar@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
- 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

# A strong **Scientific Advisory Board** is guiding us.

**Dr. Heather Olson,** Neurologist, Boston Children's; Professor Harvard Medical School

HARVARD MEDICAL SCHOOL

**Dr. Jeffrey Neul,** Scientist, Vanderbilt Kennedy Center; Professor of Pediatrics, Division of Neurology

VANDERBILT KENNEDY CENTER for Research on Human Development

**Dr. Steven Gray,** Scientist, Gene Therapy Center at UNC School of Medicine

UNC SCHOOL OF MEDICINE

**Dr. Alex Paciorkowski,** Scientist, University of Rochester Medical Center; Assistant Professor, Director

UNIVERSITY of ROCHESTER MEDICAL CENTER

**Dr. Walter Kaufmann**, Scientist and Professor of Neurology, Greenwood Genetic Center

Greenwood Genetic Center

**Dr. Gordon Fischell,** Scientist, Broad Institute; Professor, Harvard Medical School

BROAD INSTITUTE

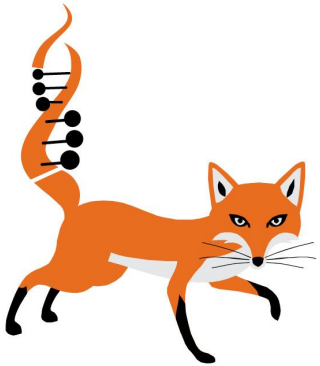
# Research Goals - what we are looking to fund

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

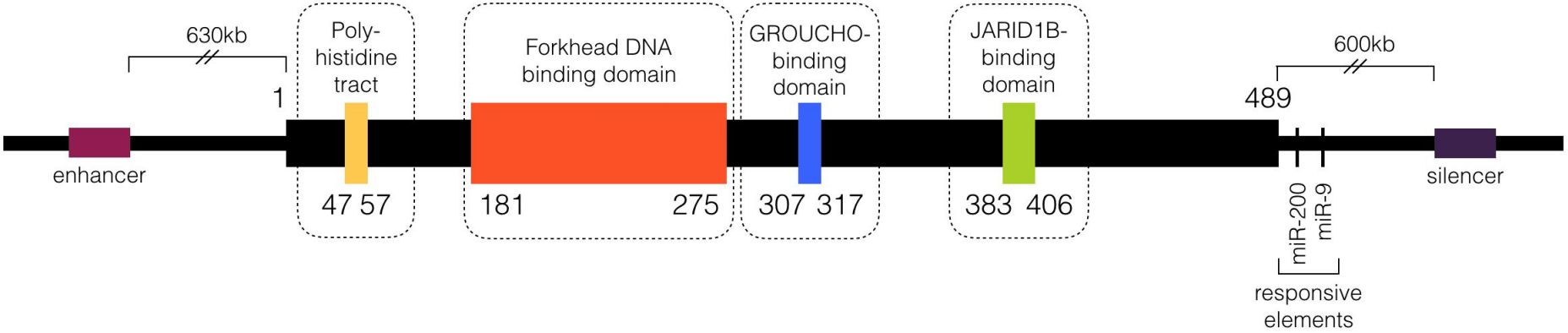
# FOXG1 Science



# *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**

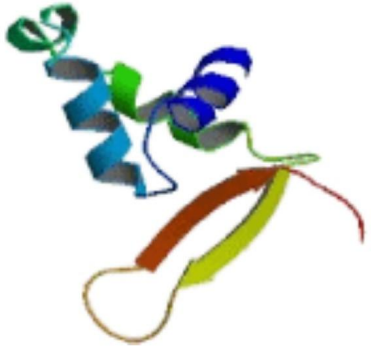
- Low muscle tone leads to reflux, constipation
- Many individuals require feeding tube placement

**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…**

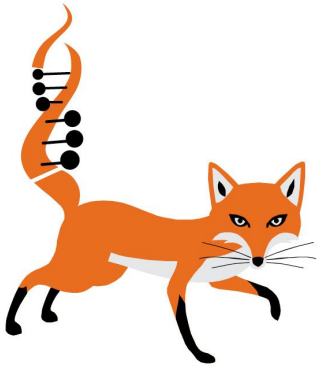
- iPSC neurons derived from patients with an autism spectrum disorder + macrocephaly (larger head size) show:
  - Overproduction of GABAergic progenitors and neurons (principle inhibitory neurons in adult brain)
  - Disrupted balance of inhibitory and excitatory neurons
  - Increased *FOXG1* expression

- 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



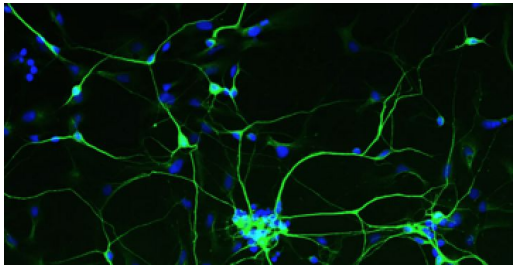
# *FOXG1* promising for research investment

**We know:**

- Affected Gene
- Strong genotype-phenotype correlation with recently published paper in **Genetics In Medicine**: *FOXG1 syndrome: genotype–phenotype association in 83 patients with FOXG1 variants 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

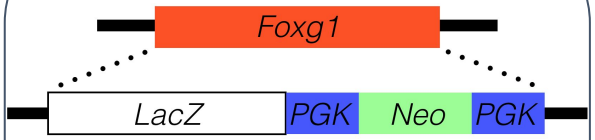


Boston Children's Hospital
Until every child is well℠

Stanford Children's Health

# Existing *Foxg1* 'knock-out' models

### ***Foxg1-Lacz* Model**

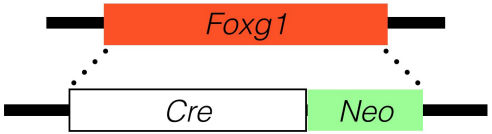



*Foxg1* replaced by a LacZ + PGK/Neo cassette

- 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**



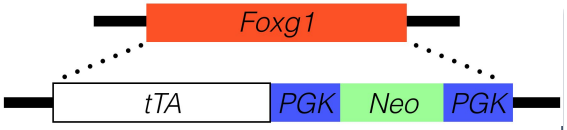

*Foxg1* replaced by a Cre/Neo cassette

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**



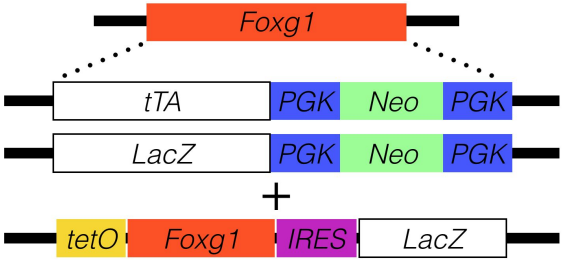

*Foxg1* replaced by a tTA + PGK/Neo cassette

- 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

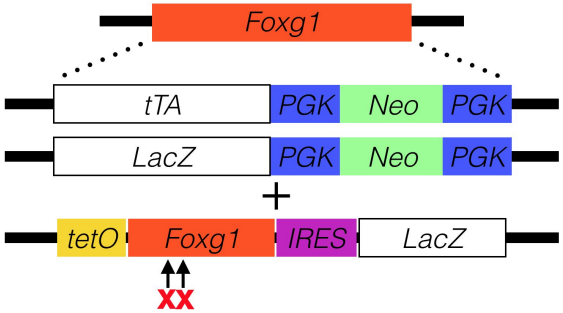
**Conditional *Foxg1* Knockout**




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

Hanashima C, *et al.* 2007

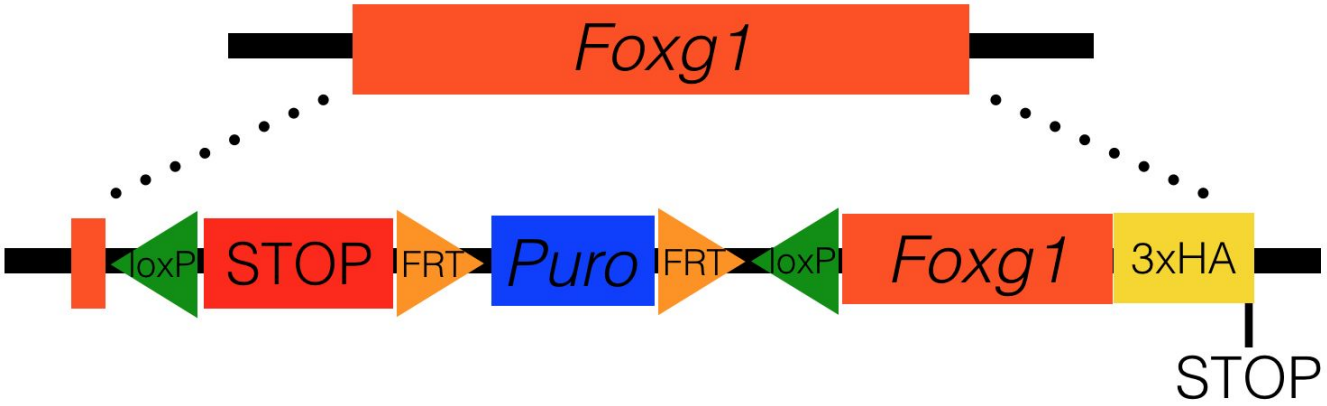
**DNA-Binding Defective *Foxg1***




- 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:

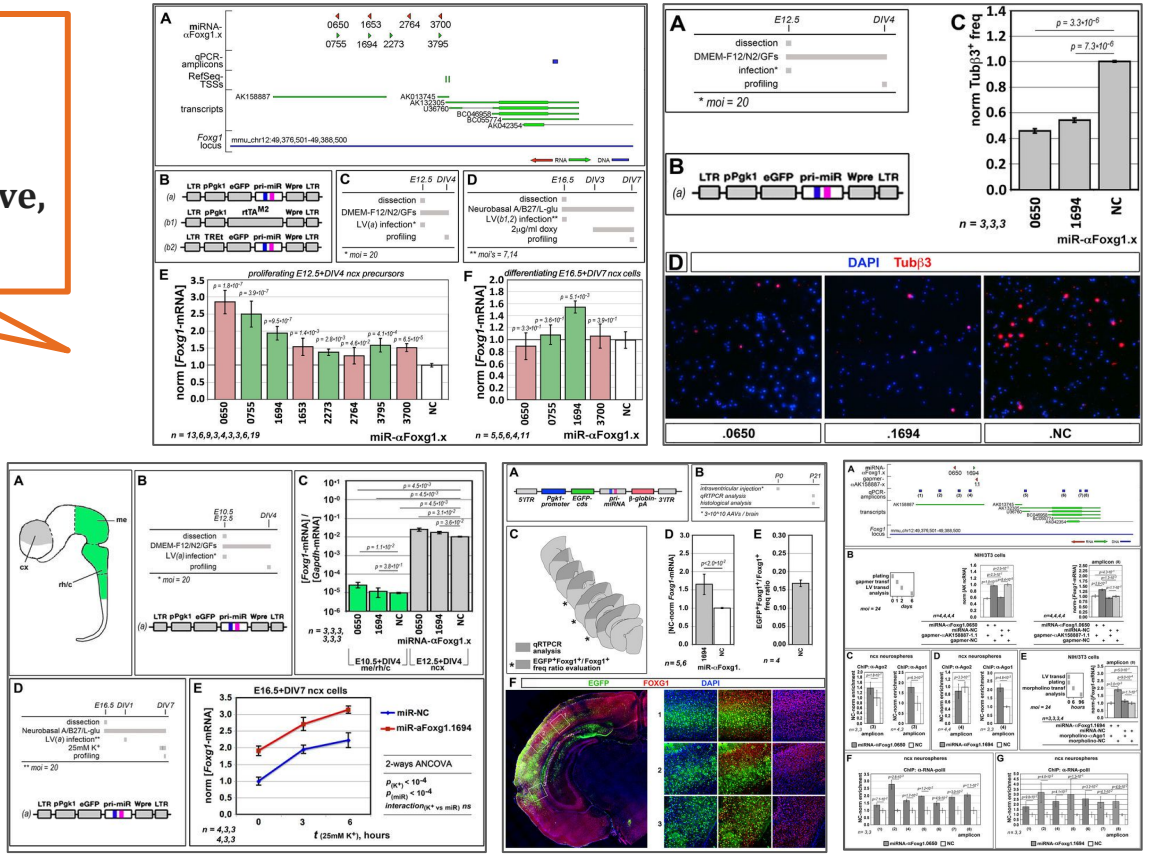
- Go to the web site http://biobanknetwork.telethon.it
- Select “login” option (on the lower left corner) and then “register”.
- Fill in the form fields (mandatory), and then confirm the registration. You will receive immediately username and password by email.
- Once you receive username and password 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**

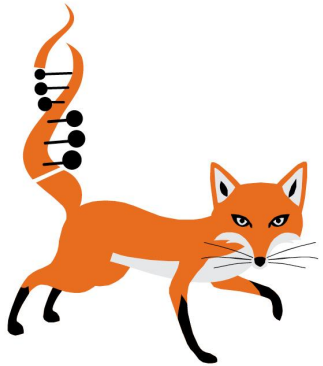
"**Remarkably, *Foxg1* was upregulated** by 1.66 ± 0.30 folds"

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



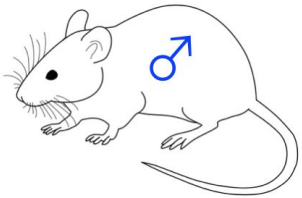
RNA activation of haploinsufficient *Foxg1*gene in murine neocortex, Cristina Fimiani, Elisa Goina, Qin Su, Guangping Gao & Antonello Mallamaci, *Scientific Reports* 6, Article number: 39311 (2016)

# Lessons from other genetic neurodevelopmental disorders



# Gene therapy stabilizes/improves symptoms in Rett syndrome mouse model

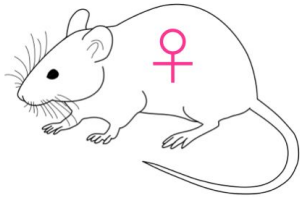
*Mecp2* stop/y
4-8 weeks



\+
scAAV9/*cre*

- Improved survival
- Robust behavioural recovery

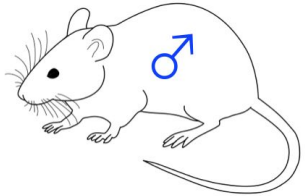
*Mecp2* stop/+
7-10 months



\+
scAAV9/*cre*

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

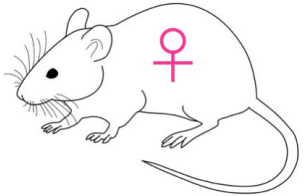
*Mecp2* null/y
4-8 weeks



\+
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.

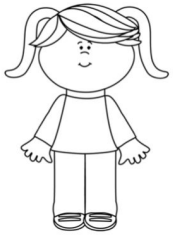
# Human recombinant IGF-1 in Rett and Fragile X syndromes

IGF-1 impacts CNS via two pathways:

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

AKT implicated in functional regulation of FOX transcription factors

*MECP2* $^{x/+}$

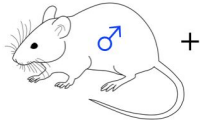


n=10
(20 wk)

\+ 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