Leiden Drug Development Conference
The future of: Humanized models

DONDERS INSTITUTE

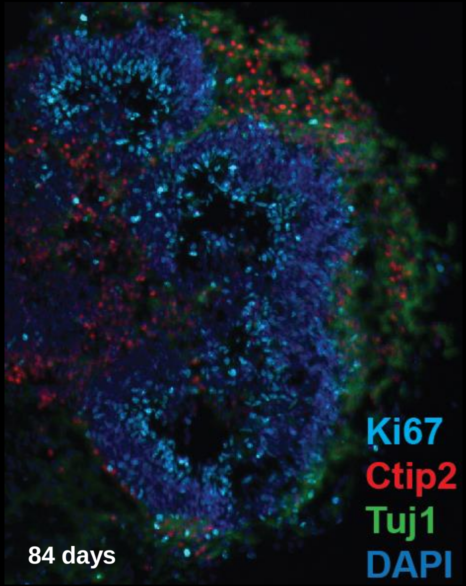
The Edge of the Possible:

Brain Organoids alongside Animal Models to study Neurodevelopmental Disorders

Sharon M. Kolk

Radboud University Nijmegen

Brain organoids to model neurodevelopmental disorders....and more



proliferating cells

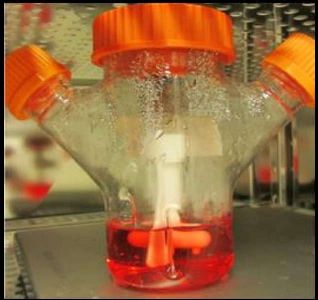
layer 5 neurons

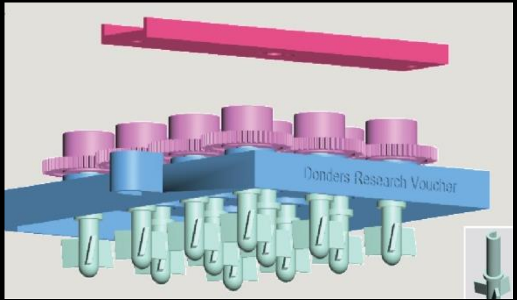
postmitotic neurons

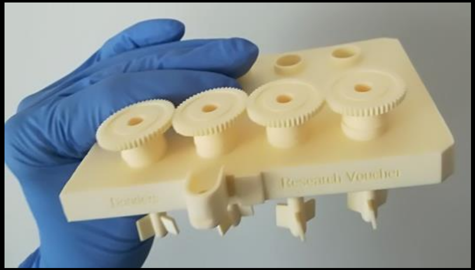
nuclei cells

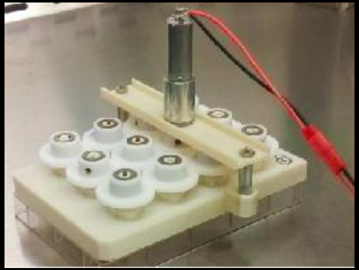
<u>Human</u> brain organoid

Scalable mini 3D-printed bioreactors to grow brain organoids (Donders Innovation Voucher)





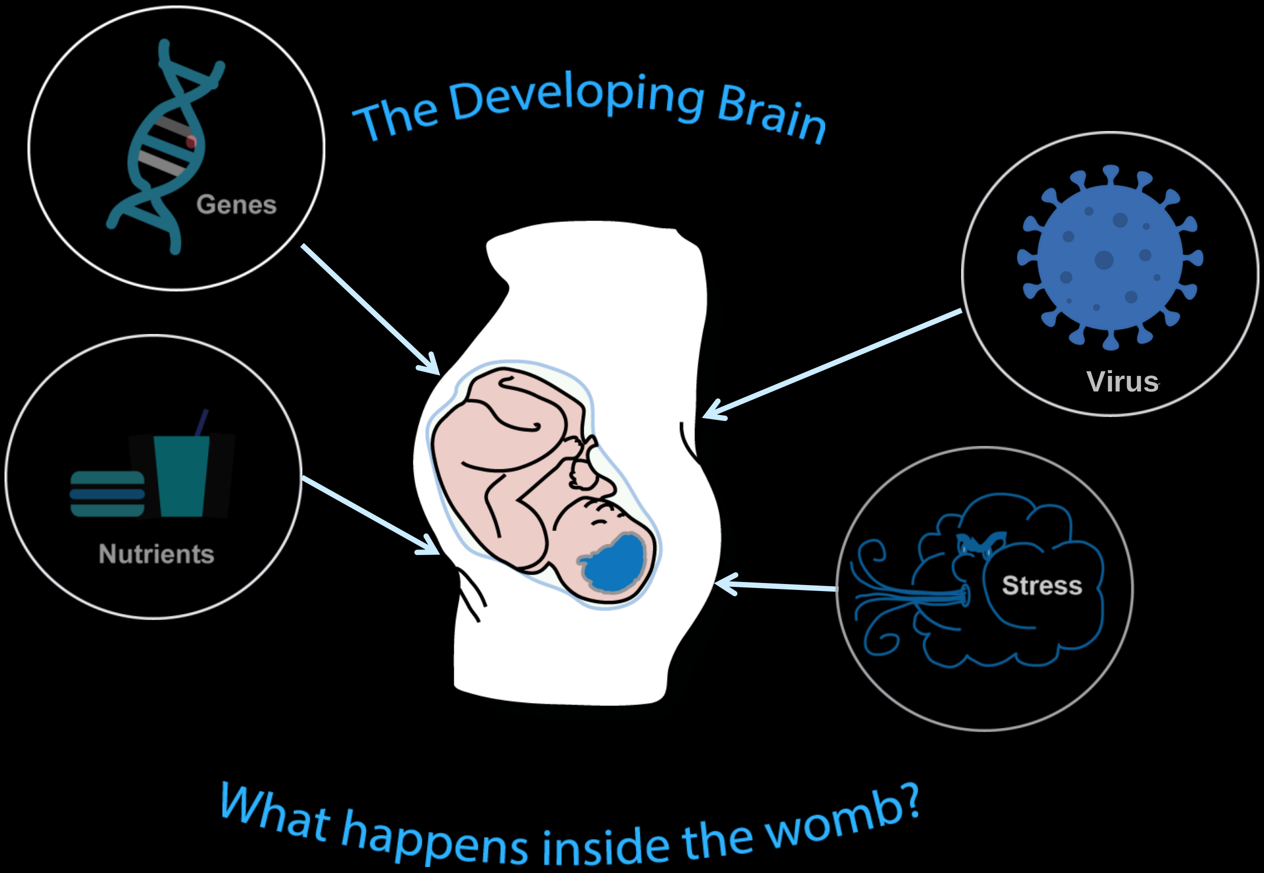




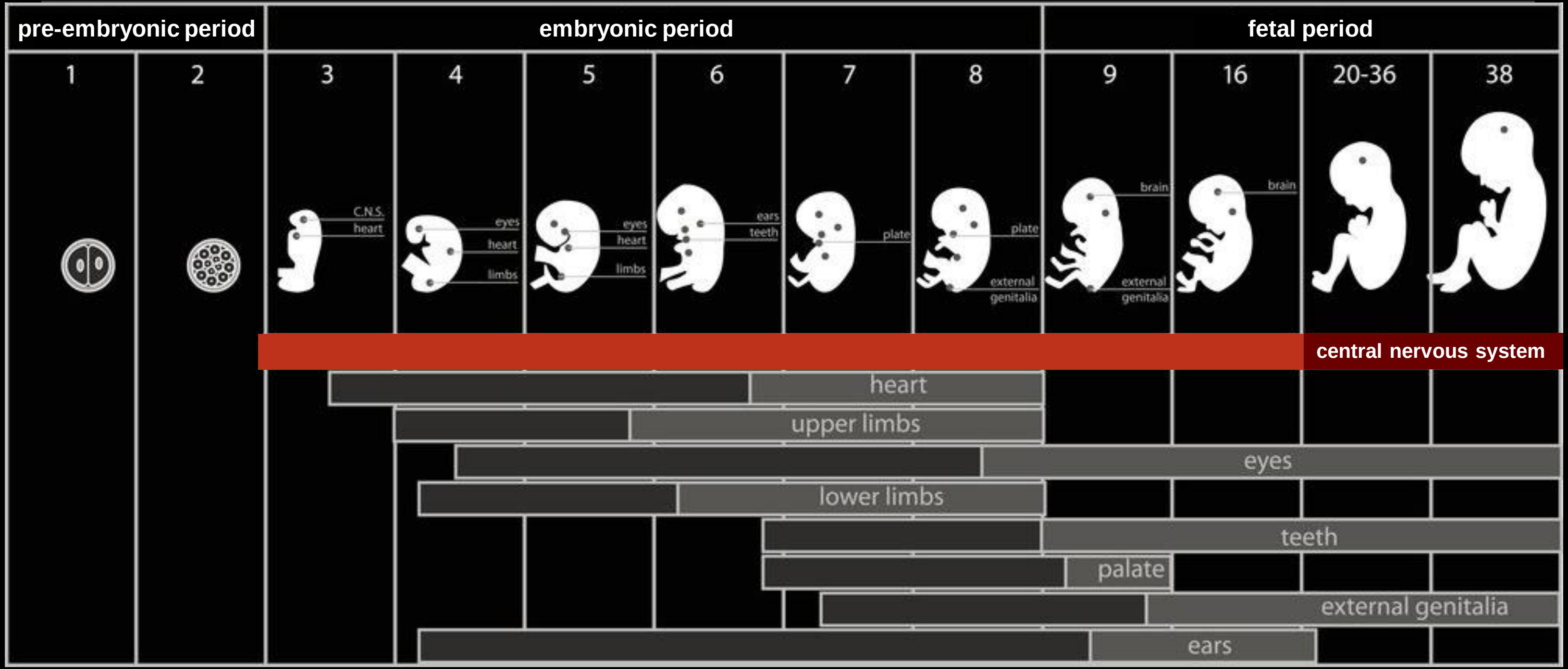
- Testing (possible) risk factors e.g. pharmaca, drugs, infections
- High-throughput set-up, easy accessible, no blood-brain barrier
- Carefully controlled variables (type/age of organoid, concentration etc)

Attractive to many domains (academic institutes, public/ private partners)

Influences on brain development



Critical window of the developing CNS

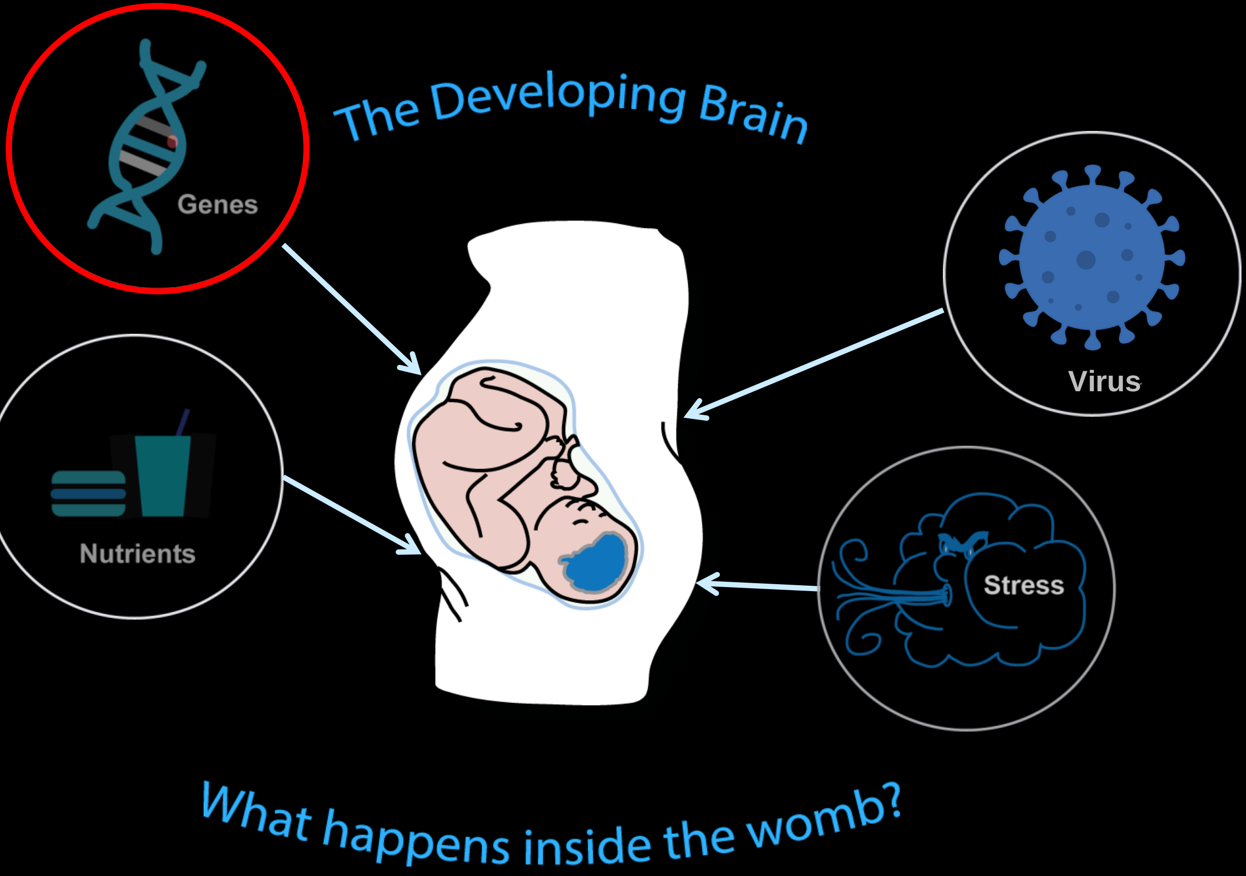


risk factors cause major birth defects

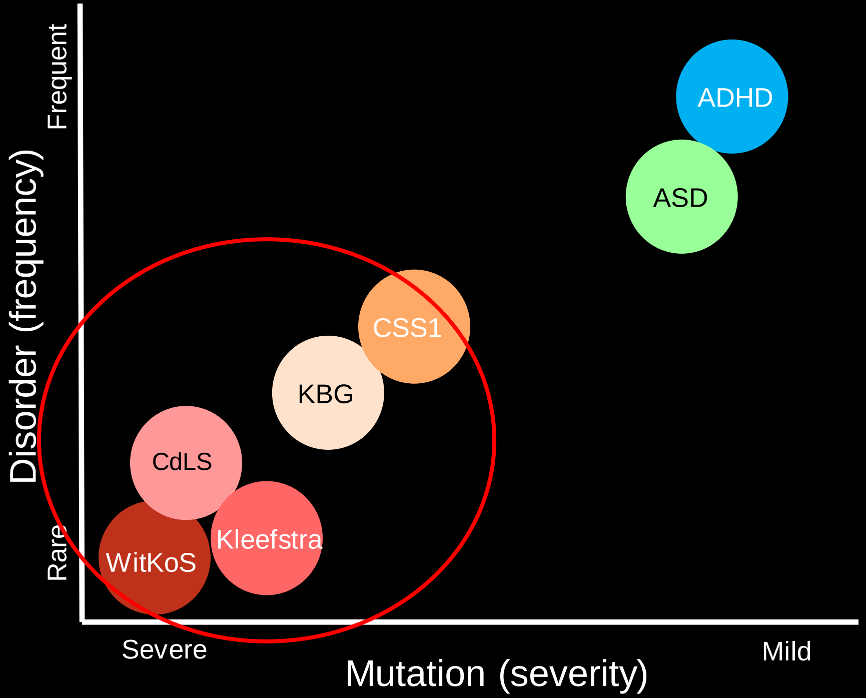
risk factors cause minor birth defects

Adapted fromSource:http://schoolbag.info/biology/humans/25.html

Influences on brain development



Neurodevelopmental disorders



Domains affected
- Emotional
- Behavioral
- Cognitive
- Communication
- Motor
- Physical health

Genes vs Environment

Adapted from Van der Voet...Schenck, Neuros. & Biobeh. Rev., 2014

WitKoS, a rare neurodevelopmental syndrome

15q24 microdeletion

frameshift mutation in *SIN3A*

Tjitske Kleefstra

OMIM entry #: 613406
WITTEVEEN-KOLK SYNDROME; WITKOS

	Present 15q24 deletion cases*				Present frameshift mutation cases #				Previous deletion cases* (Mefford et al.)		Total
Patient	1	2	3	4	5	6	7-9	10	11	12	12
Genetic defect	75,60-76,10	75,60-76,10	75,60-76,02	75,60-75,95	c.803dup; p.Leu269fs	c.1010_1013del; p.Lys337Serfs	c. 1759_1759 delT; p. Ser587fs	c.2955_2956del;p.Glu985fs	75,53-75,80	75,59-76,09	6 deletions 6 mutations
ID (TIQ<70)	+	+	+	+	+	+	+ (index), + (sister), NR (father)	+	+	-	10/12
Abnormal brain MRI	CD/CC /WM	C/CC/ WM	NP	NP	CD/WM/VD	VD	NP (index , father), NR (sister)	CC, VD	NR	CC/VD	6/8
Autism spectrum disorder (ASD)	+	-	-	-	+	+	- (index), NR (sister , father)	+	-	+	5/12
Epilepsy	-	+	-	-	+	+	- (index , father), NR (sister)	-	NR	NR	3/12

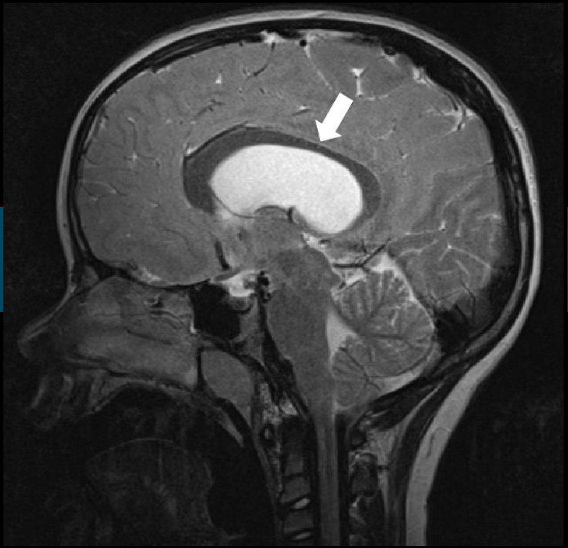
Witteveen et al., 2016, Nature Genetics

WitKoS and the neurological features

Brain MRI scans showing cortical deficits ventriculomegaly and callosal hypoplasia

early developmental events in corticogenesis

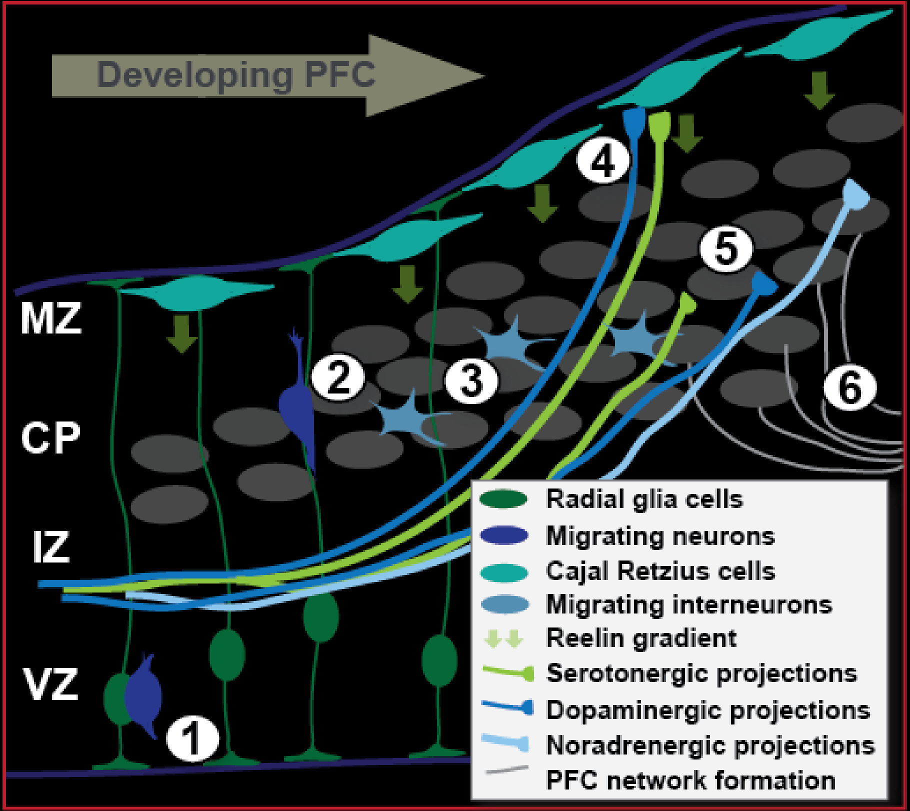
?



intellectual disability
autism
epilepsy

Witteveen et al., 2016, Nature Genetics

Corticogenesis



CP, cortical plate; IZ, intermediate zone; MZ, marginal zone; VZ, ventricular zone

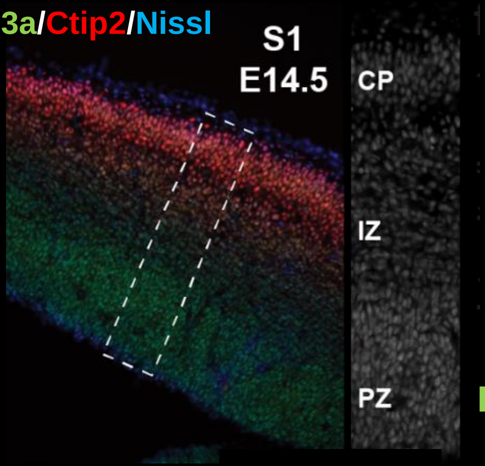
Six sites where developmental events can be hampered;

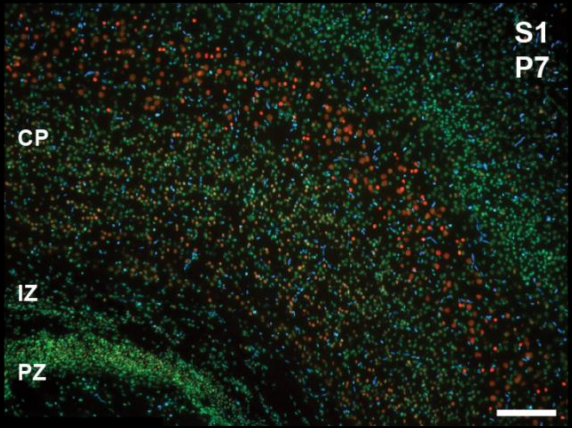
1) proliferation
2) migration
3) GABAergic interneuron integration
4) incoming projections targeting the Cajal Retzius cells
5) incoming projections coupling to the projection neurons
6) pioneer axons targeting other areas

1) Where are causative genes expressed?
2) What is their function?

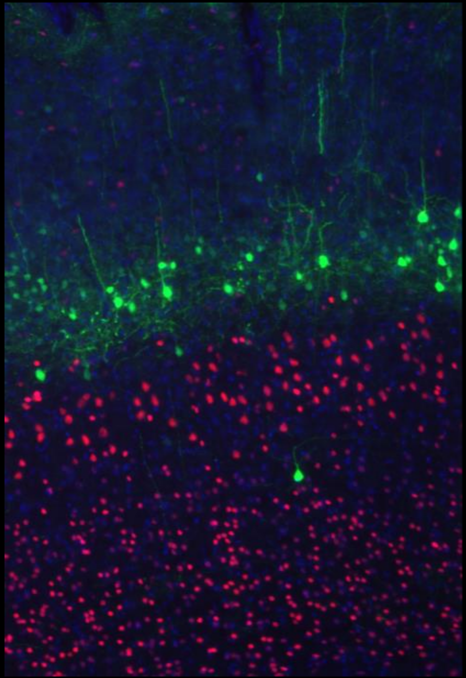
Expression and function of causative genes

Sin3a/Ctip2/Nissl

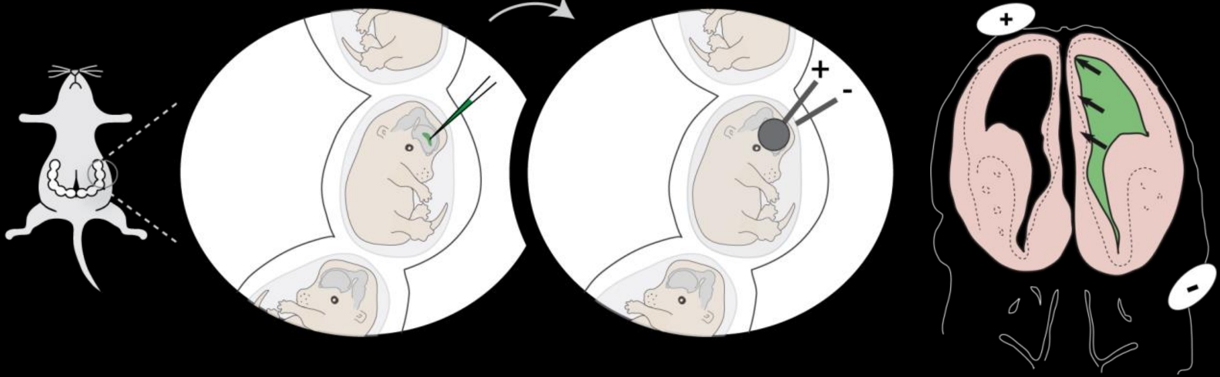




coronal mouse sections at embryonic day (E)14 and postnatal day (P)7



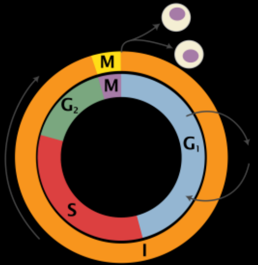
In utero electroporation (IUE) as a tool to bring down expression levels



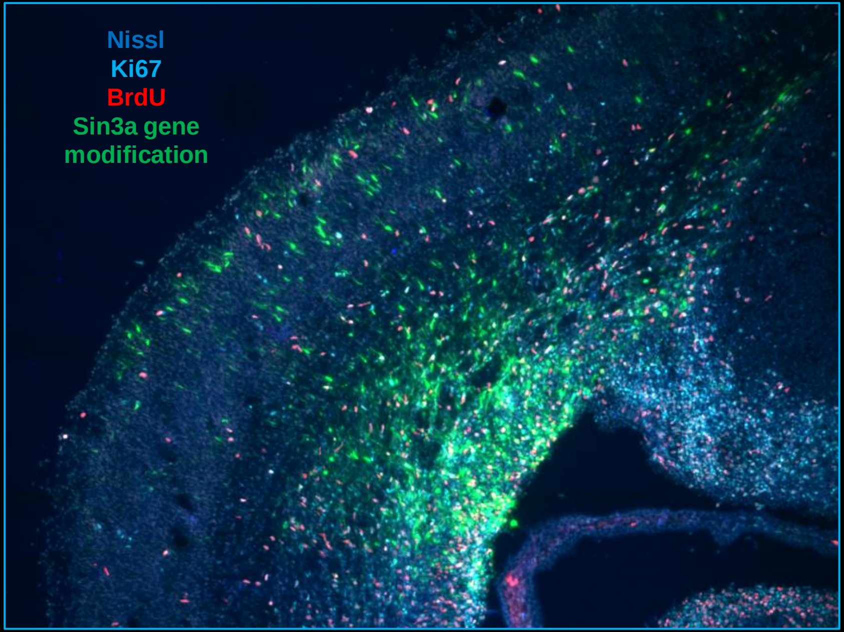
Witteveen et al., 2016, Nature Genetics

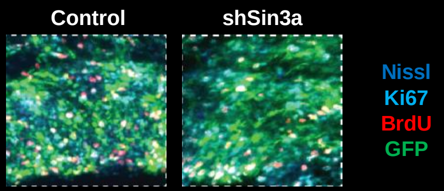
Kolk et al., 2011, FIMN

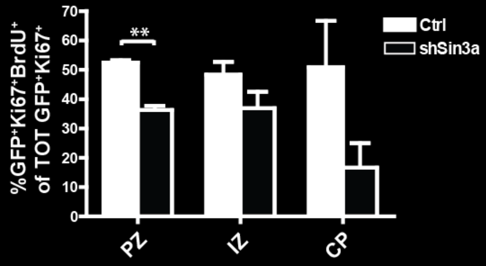
Knockdown of Sin3a decreases number of cortical progenitors

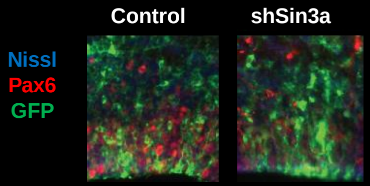


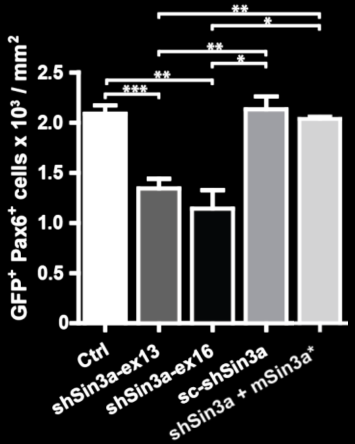
BrdU – incorporates into S-phase
Ki67 – proliferation marker
Pax6 – apical neural stem cell marker
PH3 – M-phase marker







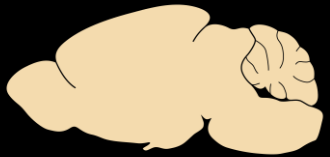




=> Follow-up

Witteveen et al., 2016, Nature Genetics

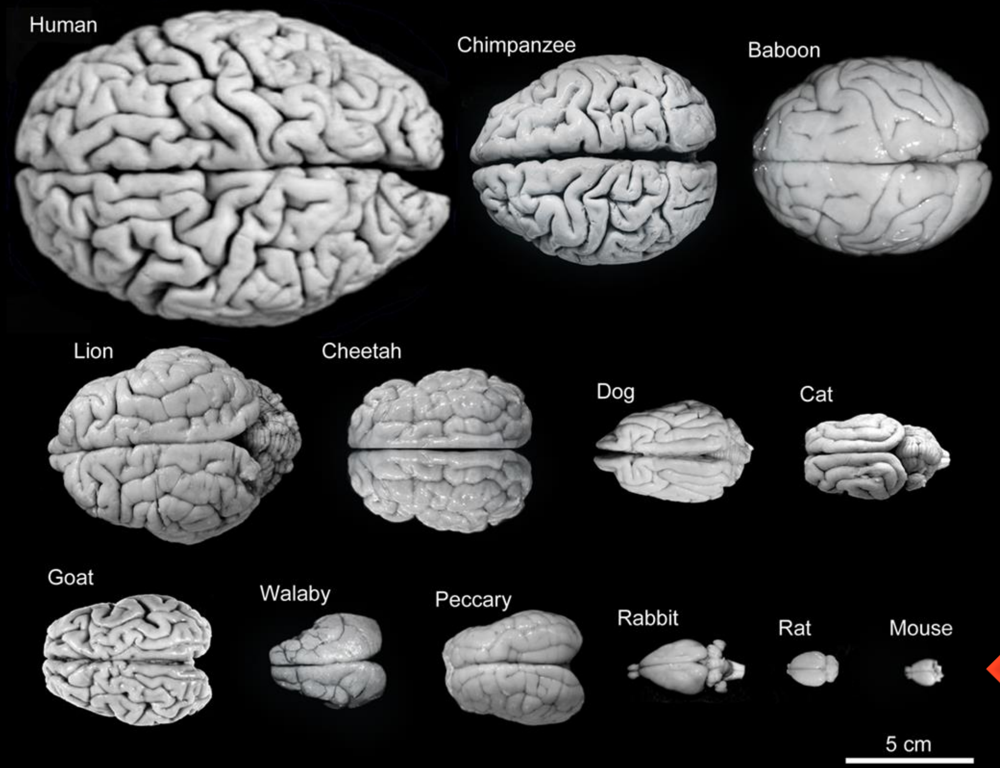
Brain evolution



Control subject WitKos patient

-Size

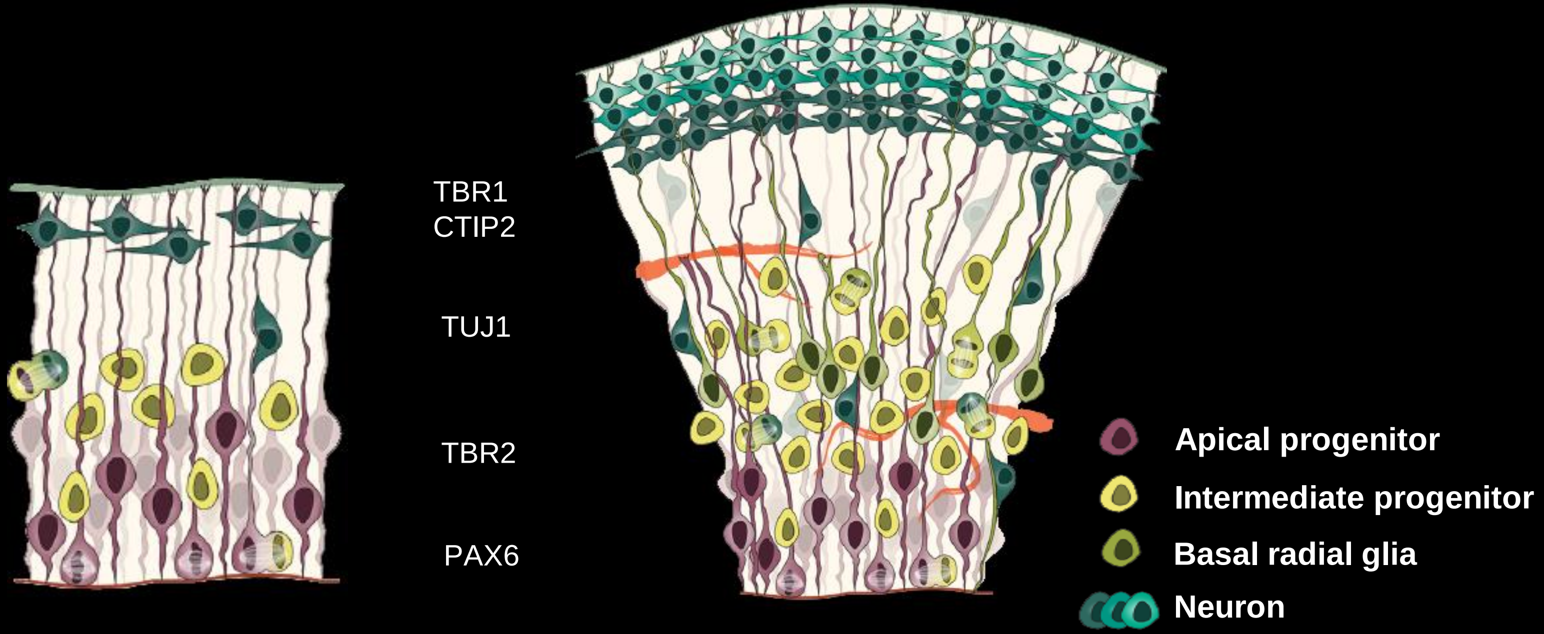
- Neuron density
- Convulsions
- Variety of neurons
- Other/<u>more</u> neurodevelopmental events



Adapted from De Felipe, Frontiers in Neuroanatomy, 2011

Mouse and human corticogenesis

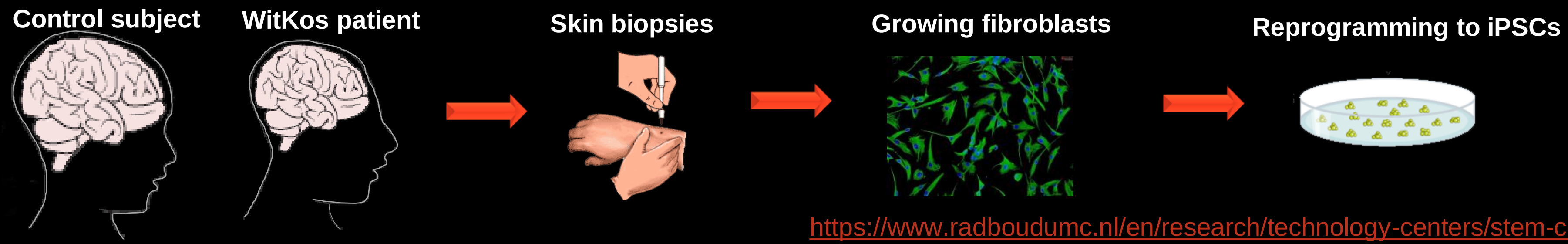
Mouse brain **Human brain**



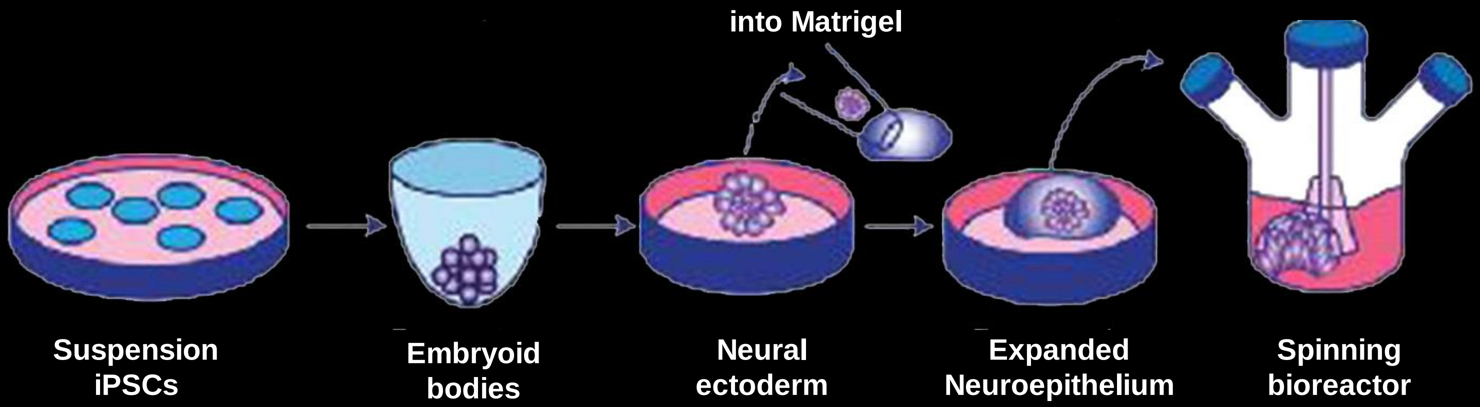
How to study molecular and cellular aspects of human brain development?

Adapted from Kelava and Lancaster, Dev Biology, 2016

Modeling monogenic NDDs such as WitKos Syndrome

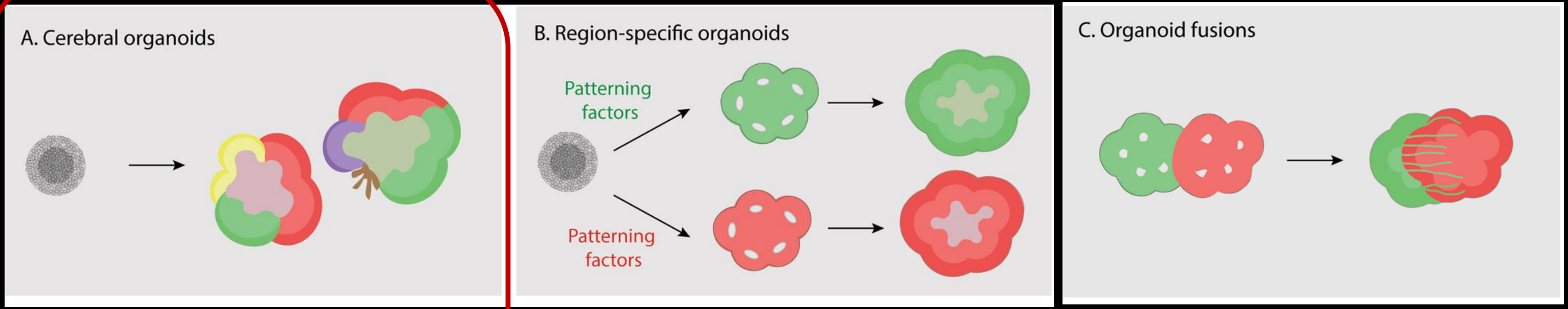


https://www.radboudumc.nl/en/research/technology-centers/stem-cells



Lancaster et al. Nature 2013

Various flavours of brain organoids



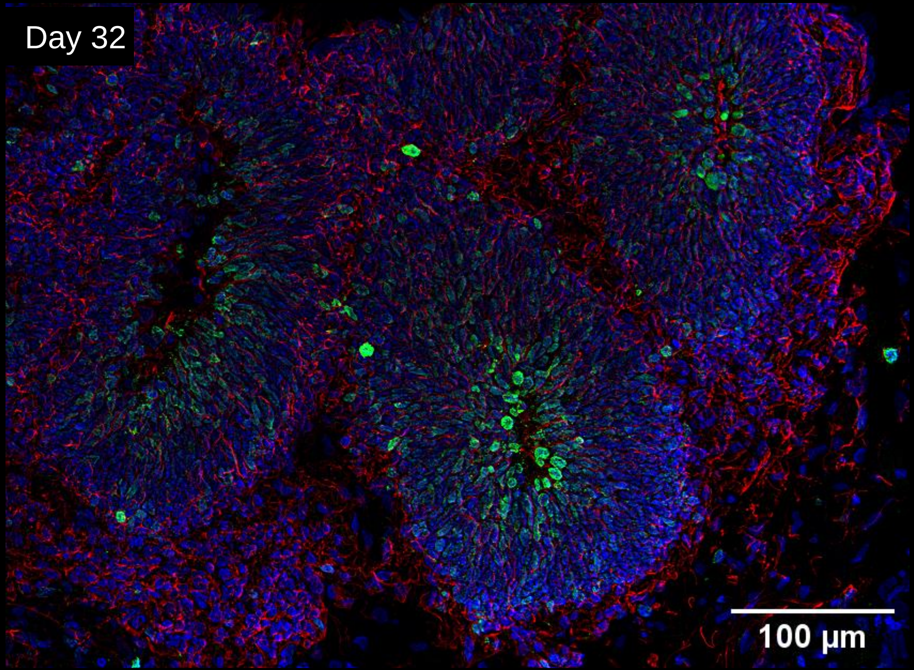
Cerebral/unpatterned

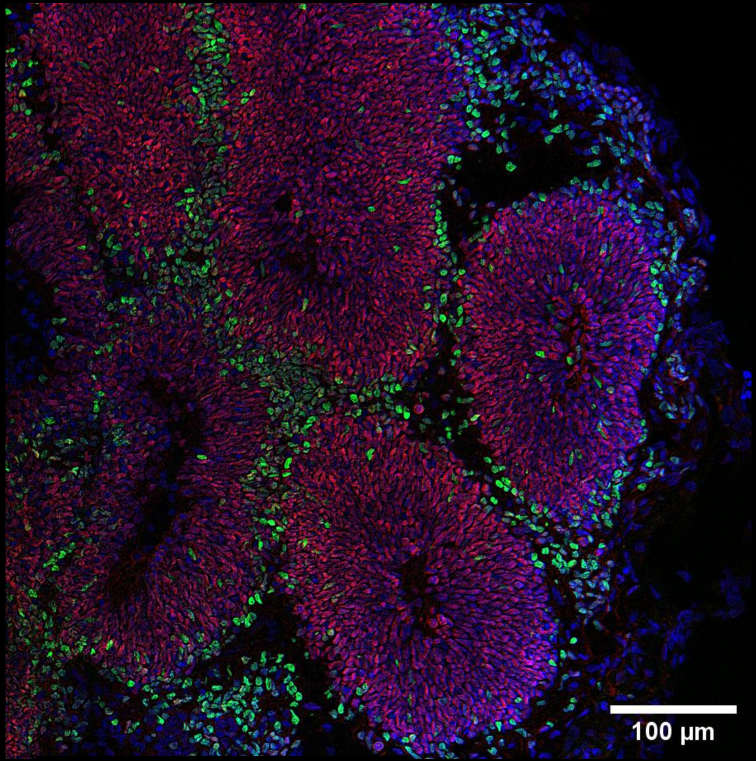
Cortical/patterned

Assembloids

Sidhaye and Knoblich 2020

Cerebral organoids to model NDDs



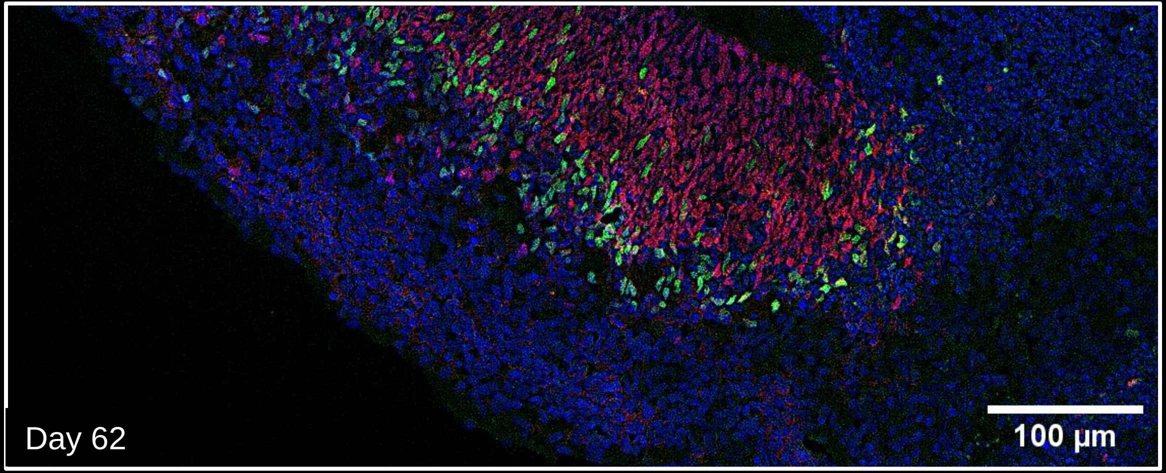


DAPI: cell nuclei
Ki67: proliferation
TUJ1: immature neurons

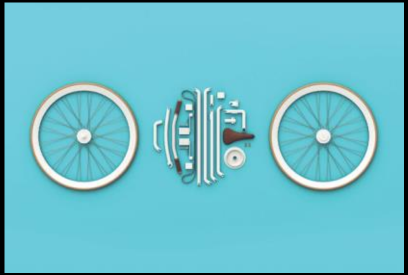
DAPI: cell nuclei
CTIP2: early cortical plate
PAX6: neural progenitors

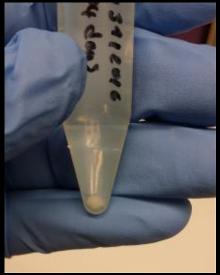
Images deconvolved with Huygens software (SVI)

Similar neurodevelopmental events during development, but...



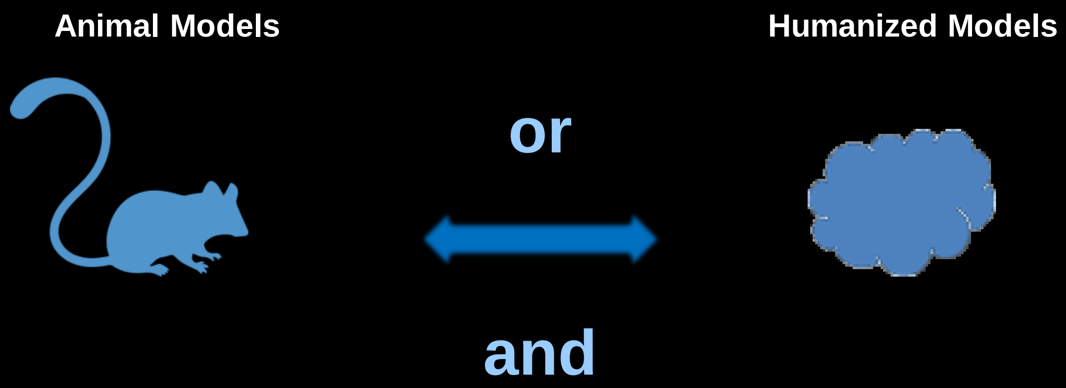
DAPI: cell nuclei
TBR2: intermediate progenitors
PAX6 : neural progenitors





The Edge of the Possible:

Brain Organoids alongside Animal Models to study Neurodevelopmental Disorders



Thanks to...

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- Danielle Vonk

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- Woo-Yang Kim

University of Bonn, Germany

- Sandra Blaess

All participating patients and families

NWO

CAPES

NIH National Institutes of Health

STEMCELL™ TECHNOLOGIES

MARIE CURIE ACTIONS

nationale wetenschaps agenda NWO

Scientific Volume Imaging
Deconvolution - Visualization - Analysis