

Disease modelling and therapeutic testing- Potential implications for SLC6A1 research



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Reasons for *in vitro* modelling on neurological diseases

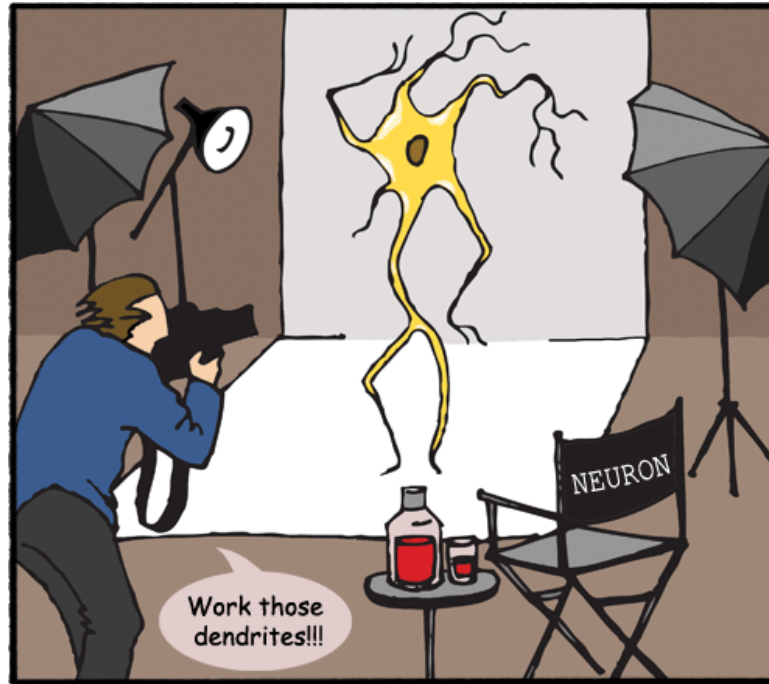
- Neurological diseases: Difficult to study
- Access to tissue/patients limited: post-mortem
- Complex phenotype. Not always genotype-phenotype correlation

***In vitro* modelling allows us to understand physiological pathways, cell interactions and get close to the pathophysiology of different diseases**

Non-cell autonomous component of neurological diseases



Astrocytes



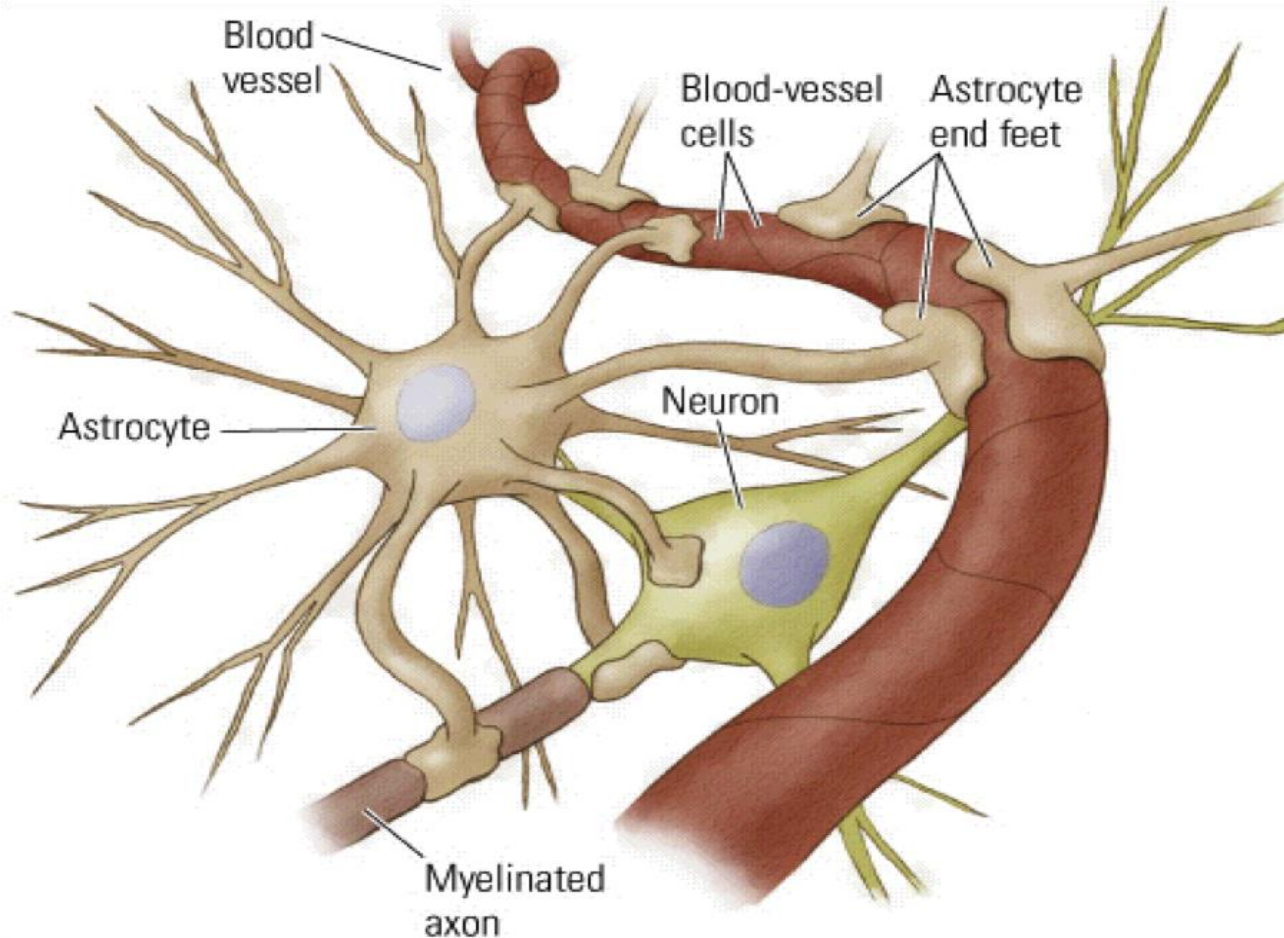
Oligodendrocytes

Microglia

http://www.clontech.com/CA/Support/Clontech_Cartoons/2013/September_2013

Neuron disease phenotype and progression is influenced by other cell types

Why are Astrocytes important?



- Neurosupportive
- Vasomodulation
- Part of Blood-Brain barrier.
- Regulation of: Glucose, neurotransmitters, Ions.

Amyotrophic Lateral Sclerosis



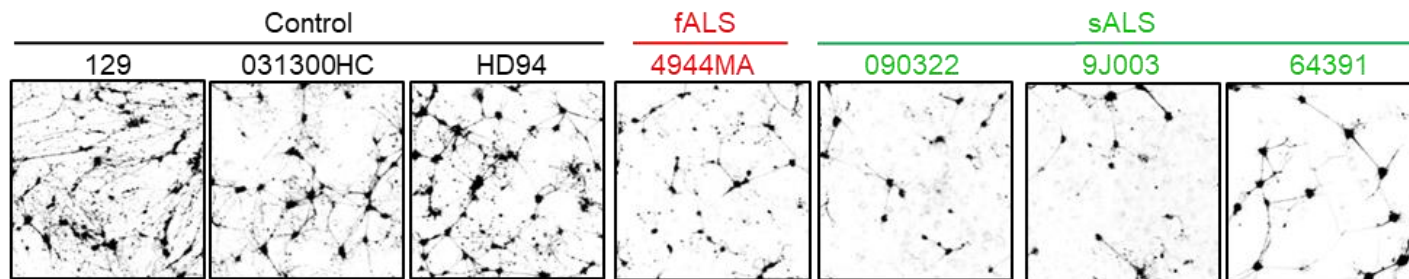
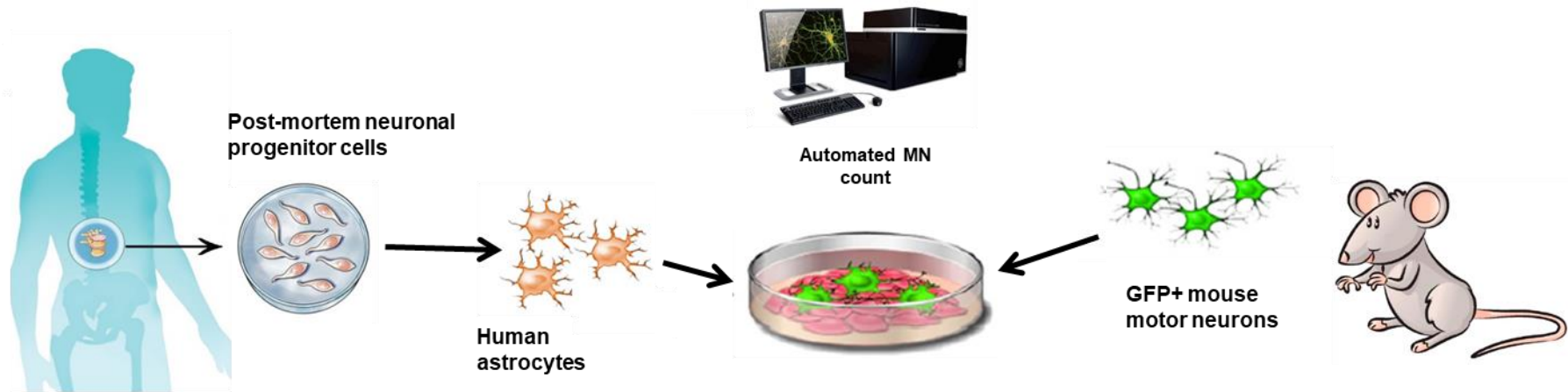
- Adult onset neurodegenerative disease
- Approx. 20'000 individuals in USA
- Degeneration of Motor Neurons
- Paralysis and ultimately death
- ~ 90% sporadic, 10% familial cases
- Various disease causing genes identified

<https://alsnewstoday.com/>

Vast Majority of cases: cause unknown

How do we study this disease?

Cell culture model using human post-mortem cells



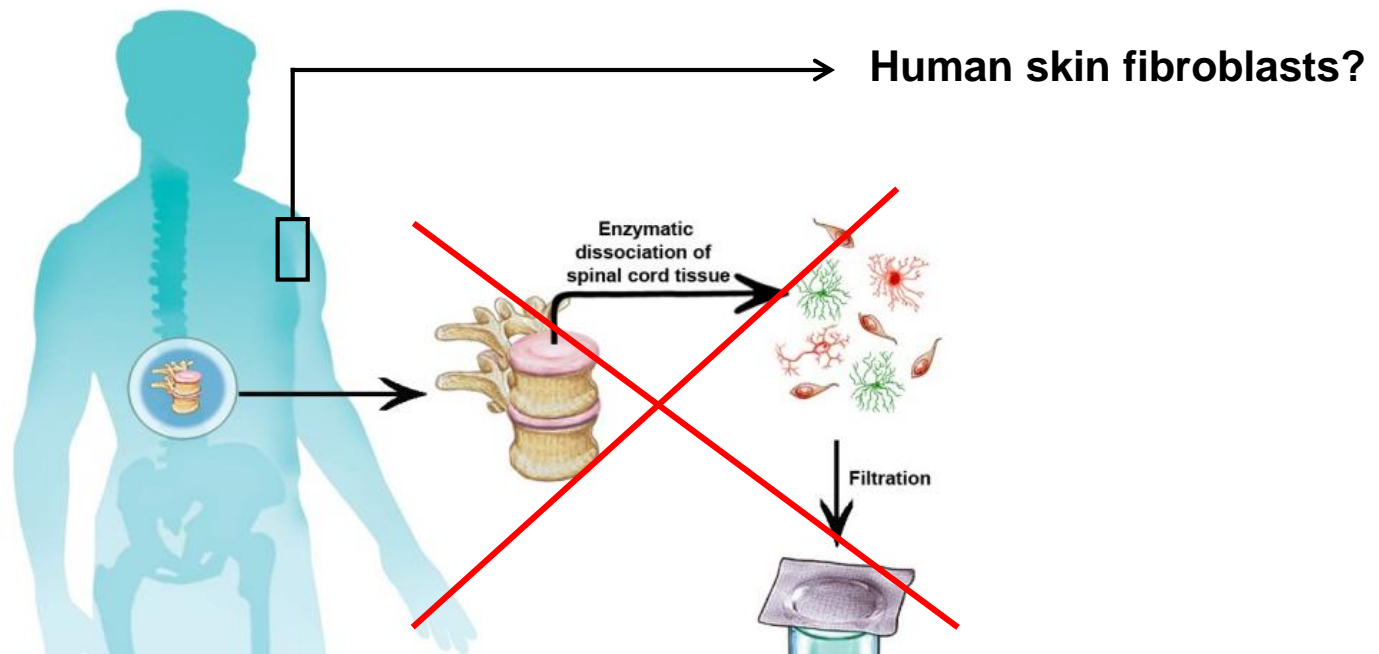
Haidet-Phillips et. al, Nat Biotech 2011 + additional age matched controls

Astrocytes from human ALS patients are toxic to motor neurons

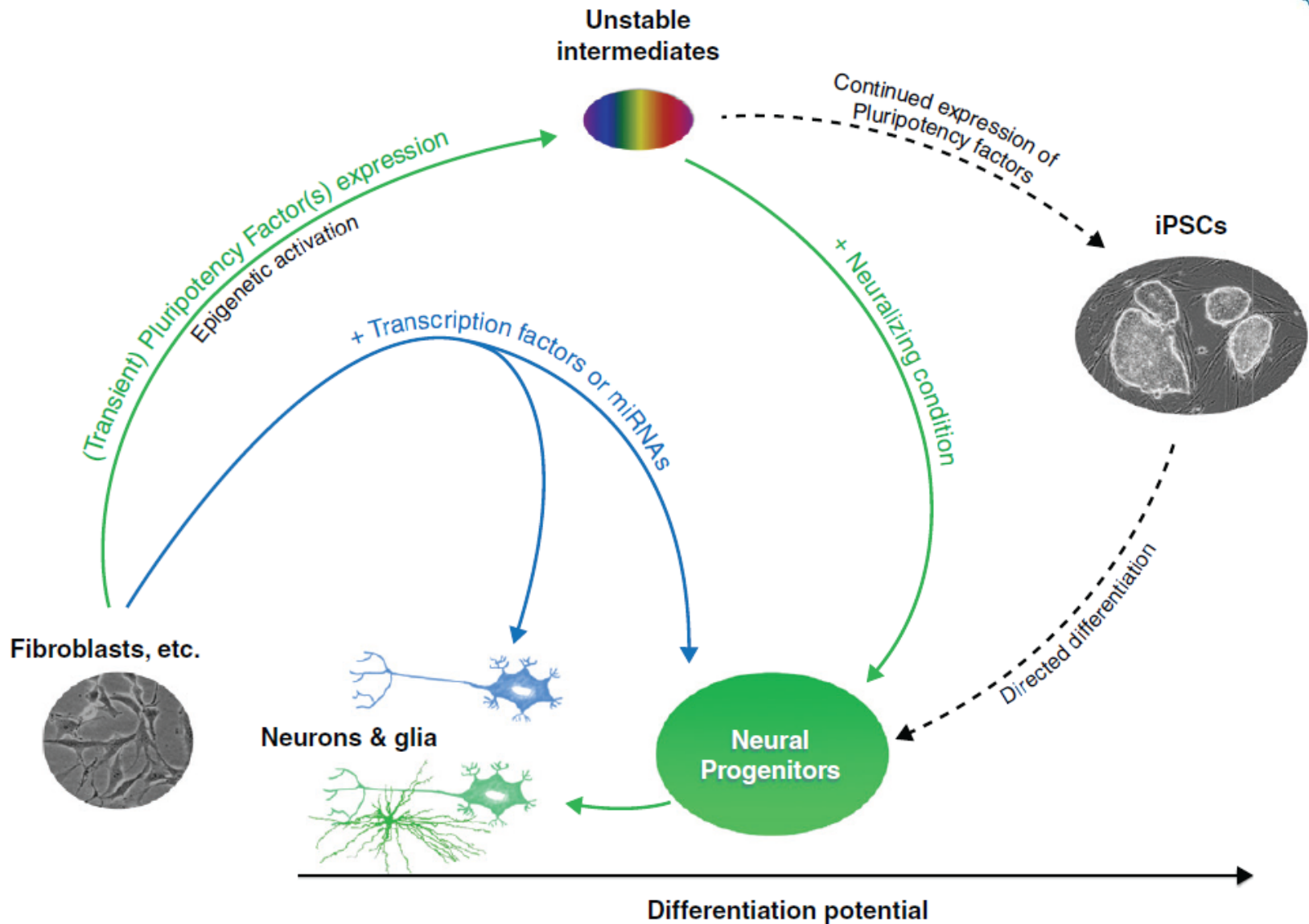
Post mortem astrocytes are a limited source



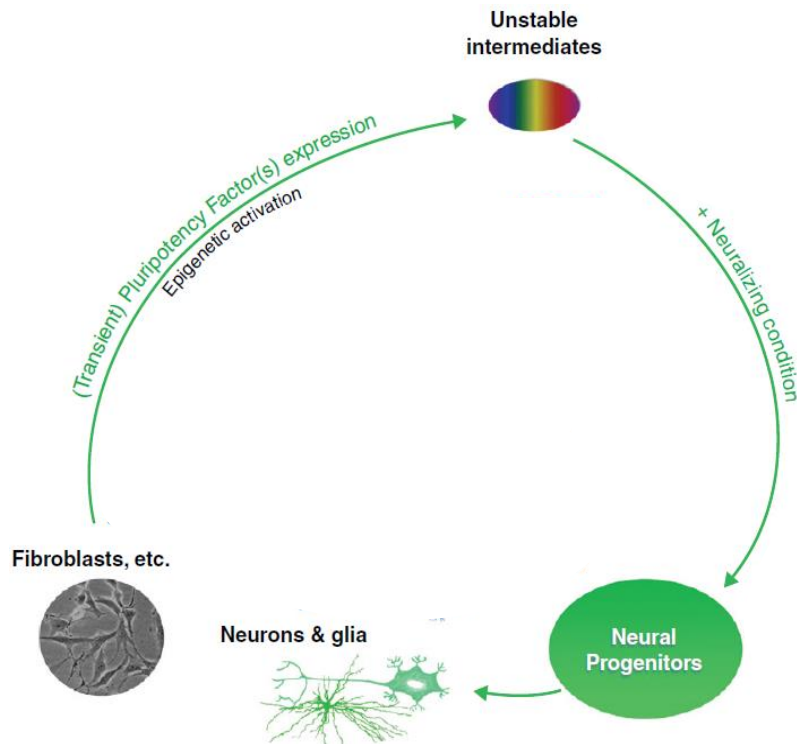
- Post-mortem samples = limited availability + represent only end-stage of disease
- No gain for the donor



Current reprogramming methods in use

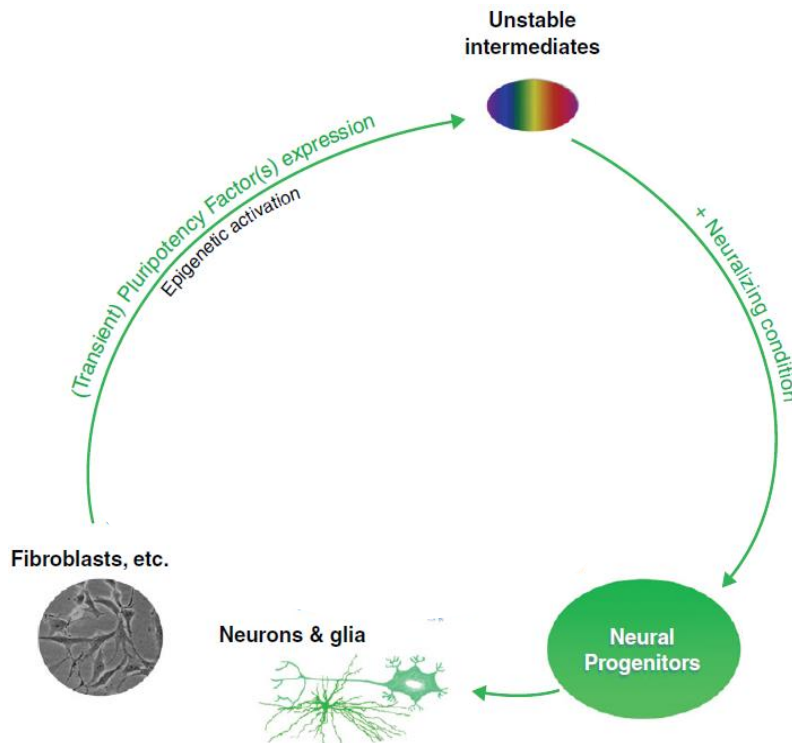


Advantages of direct conversion compared to classical reprogramming



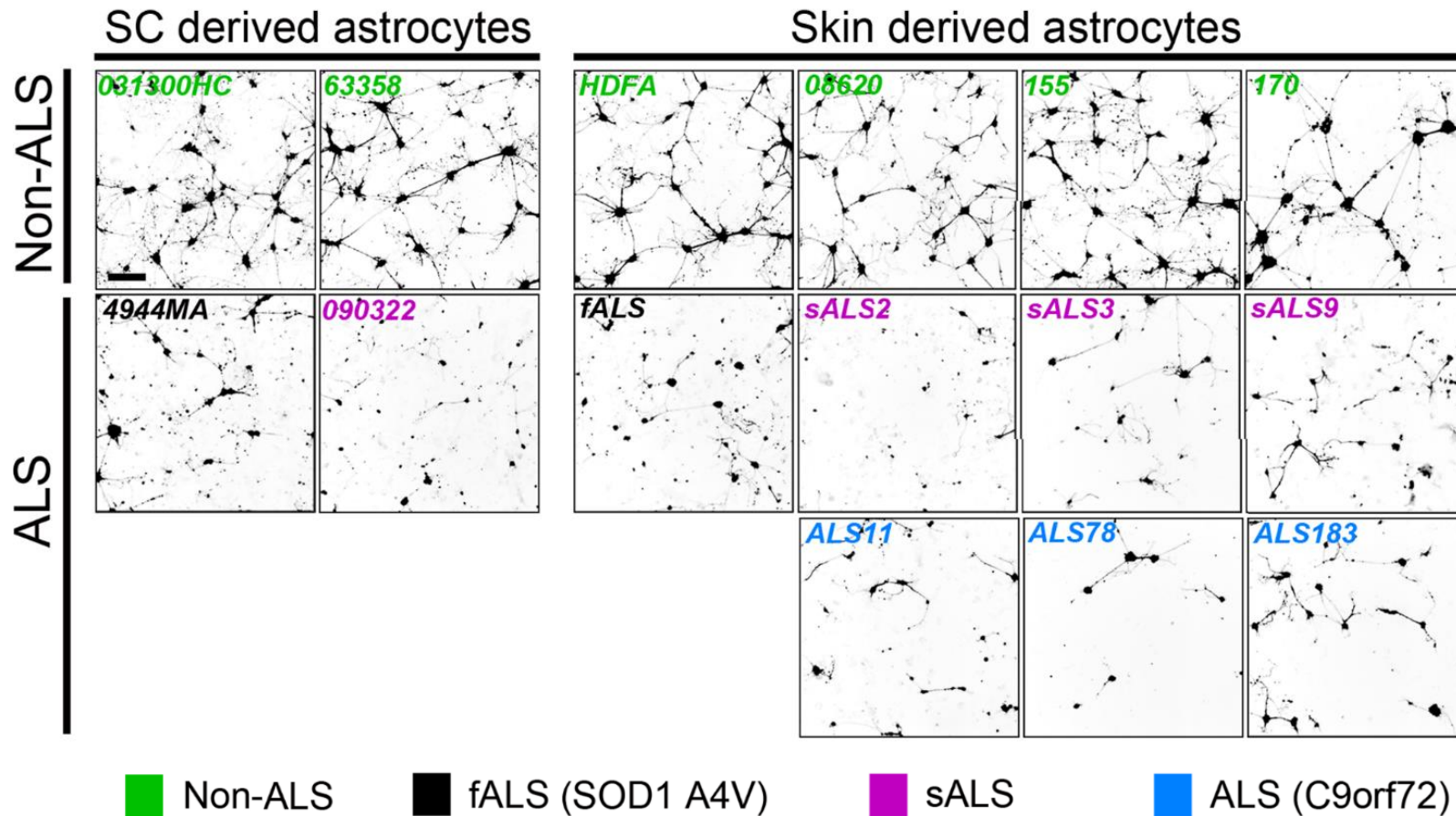
- Direct conversion is much faster
- No clonal selection – no clonal variation
- Maintenance much less time consuming
- Able to generate several disease relevant cell types (Neurons, Astrocytes, Oligodendrocytes) in parallel from the same source

Advantages of direct conversion compared to classical reprogramming

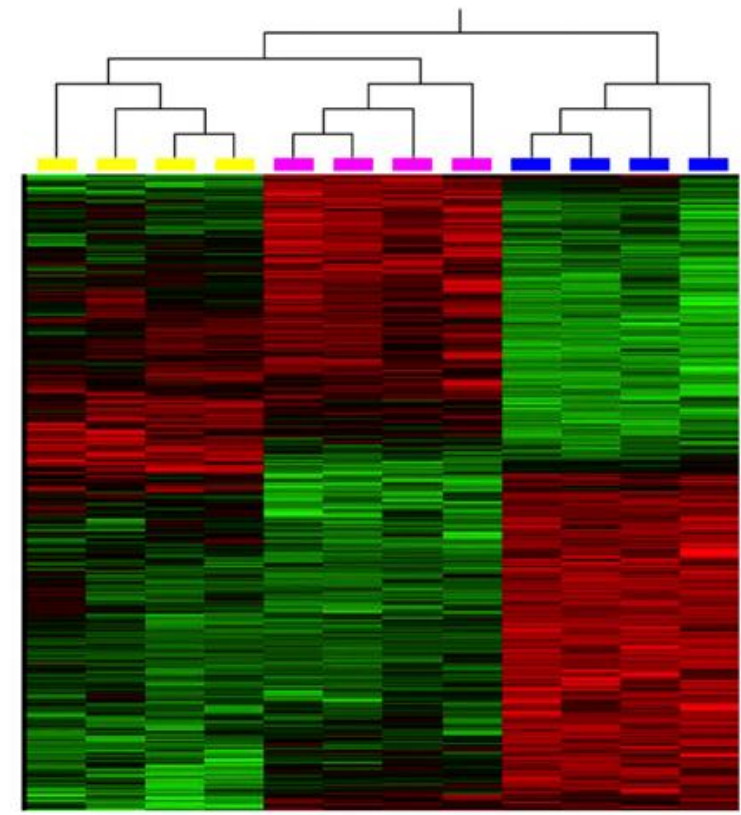
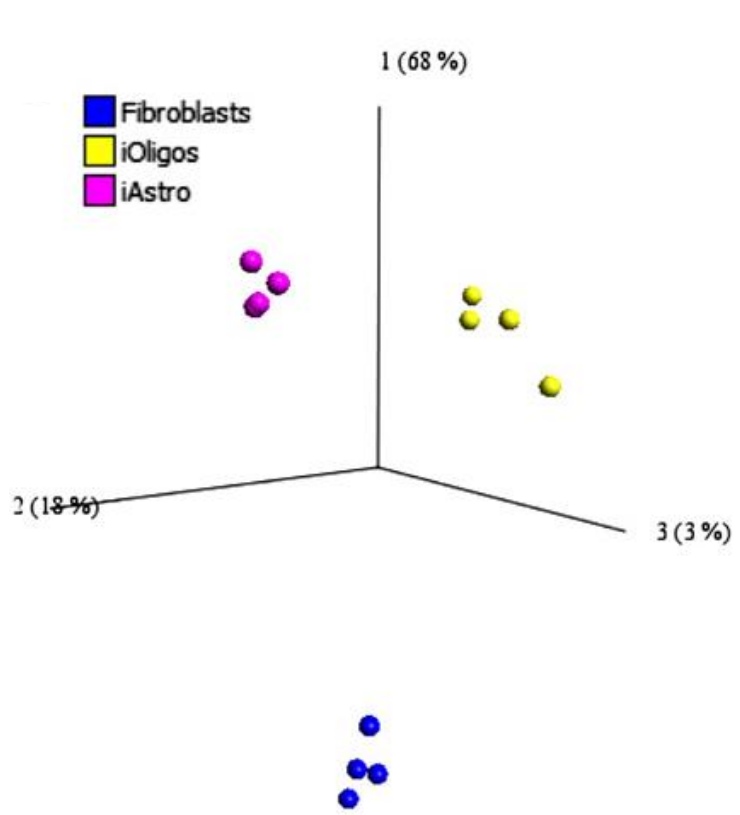


- Reprogrammed cells can be used on different assays
 - Co-Culture model system
 - Metabolic assays

iAstrocytes are similarly toxic to motor neurons compared to primary cells



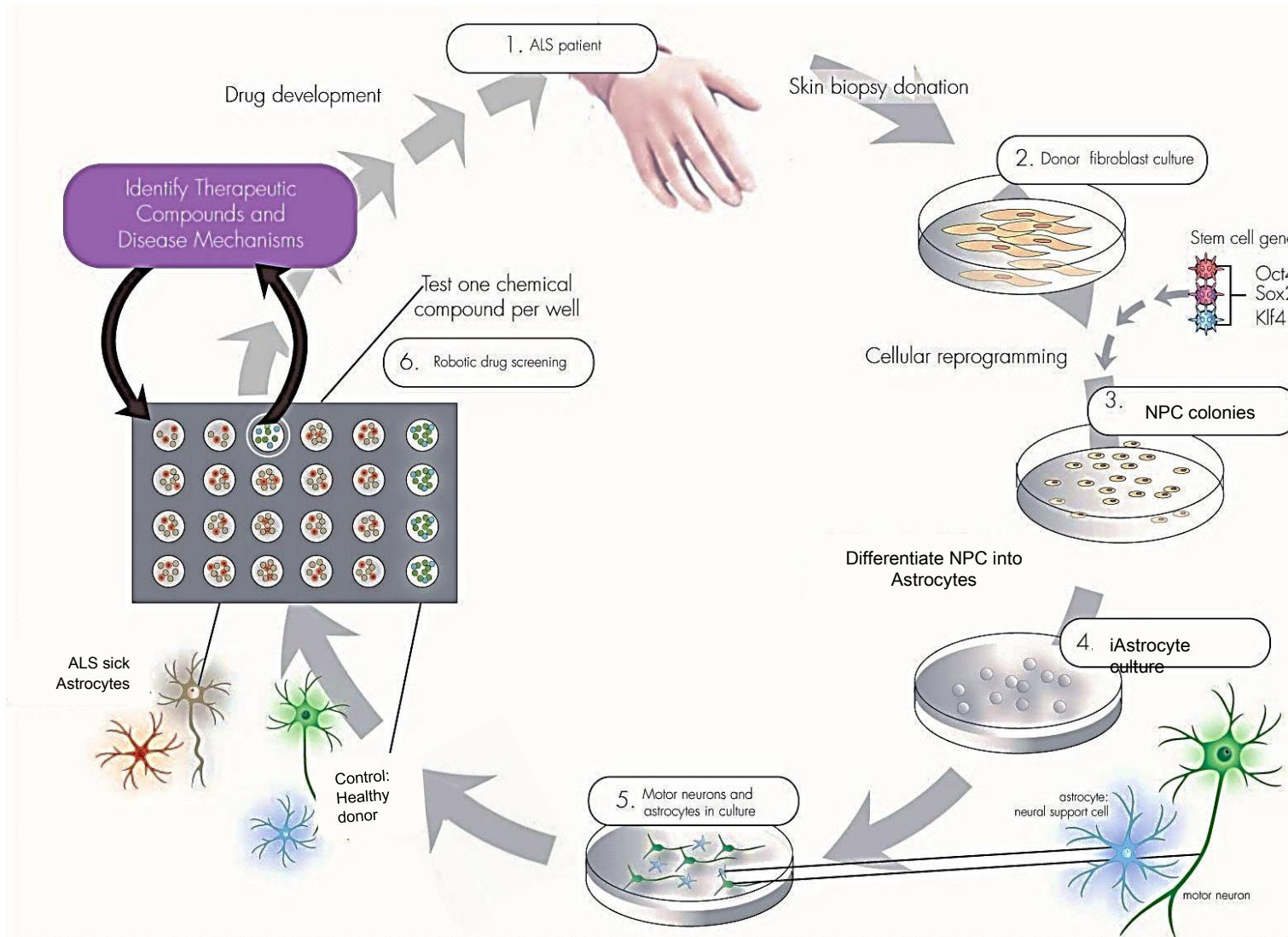
Astrocytes and Oligodendrocytes derived with this method express cell type specific genes



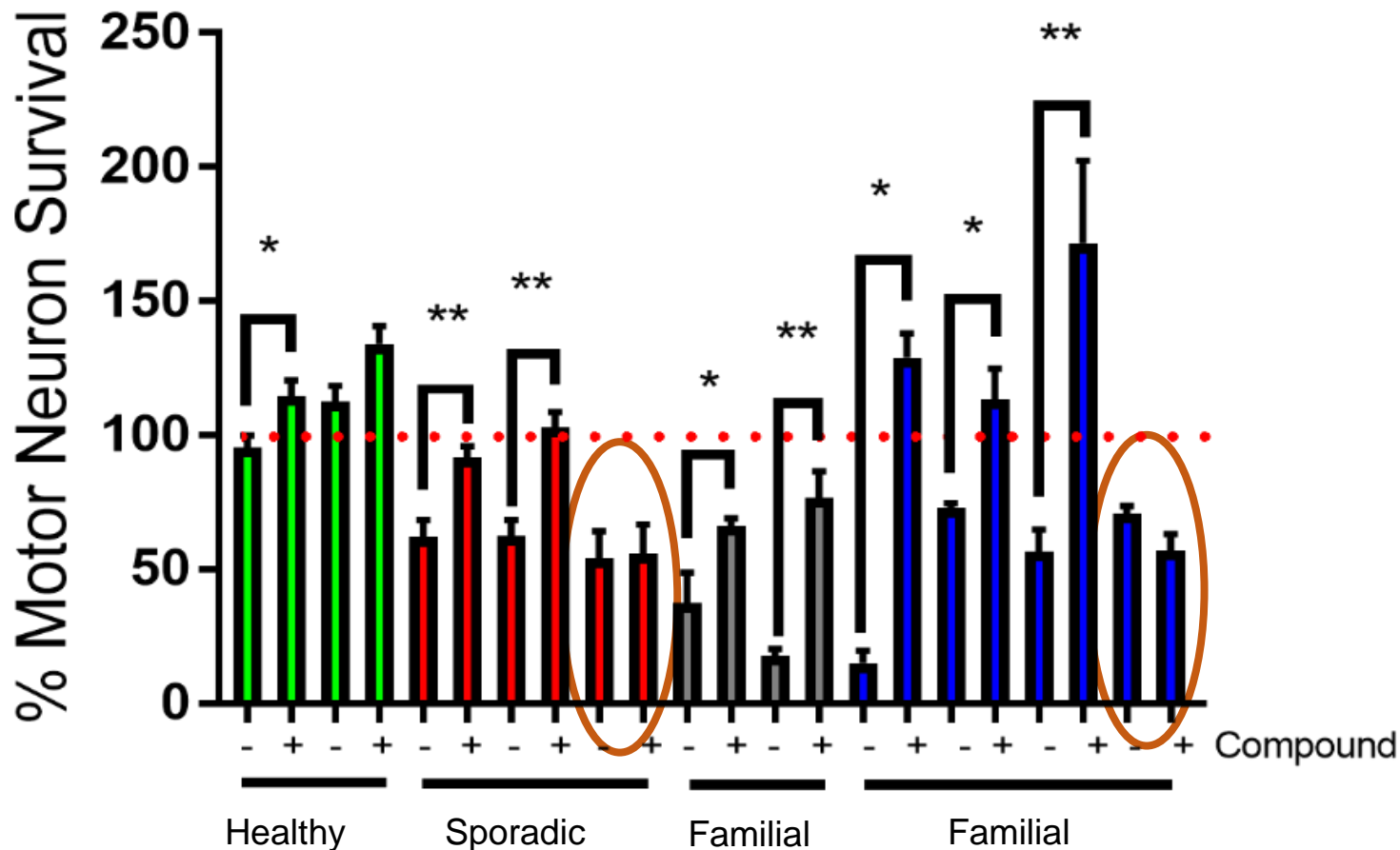
Ferraiuolo et al., PNAS 2016

Microarray RNA sequencing shows different expression patterns between primary fibroblast lines and the astrocytes and oligodendrocytes differentiated from them.

Converted fibroblasts are used to model neurological disease and drug screenings



Patient subpopulations react differently to treatments



Identification of responders and non-responders to select optimal treatment option for each patient

Expanding the model system to different diseases



- Currently more than 50 different cell lines from various neurodegenerative and neurological disorders with mutations and different disease courses:
 - Lysosomal storage diseases (Batten disease)
 - Neurodevelopmental diseases (Rett Syndrome)
 - Ion channelopathies (SCN2A)
- Disease controls, sibling controls and age matched healthy controls

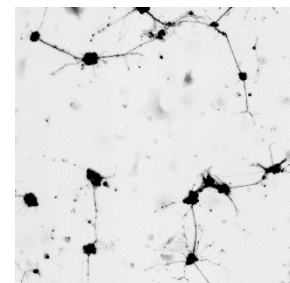
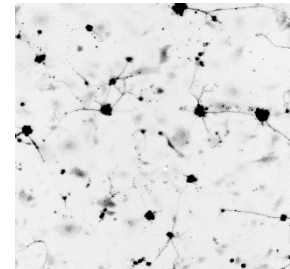
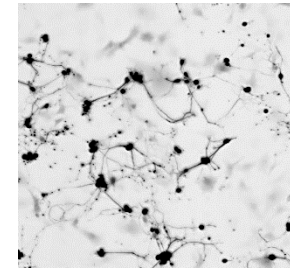
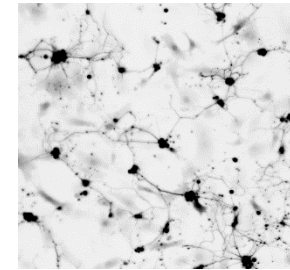
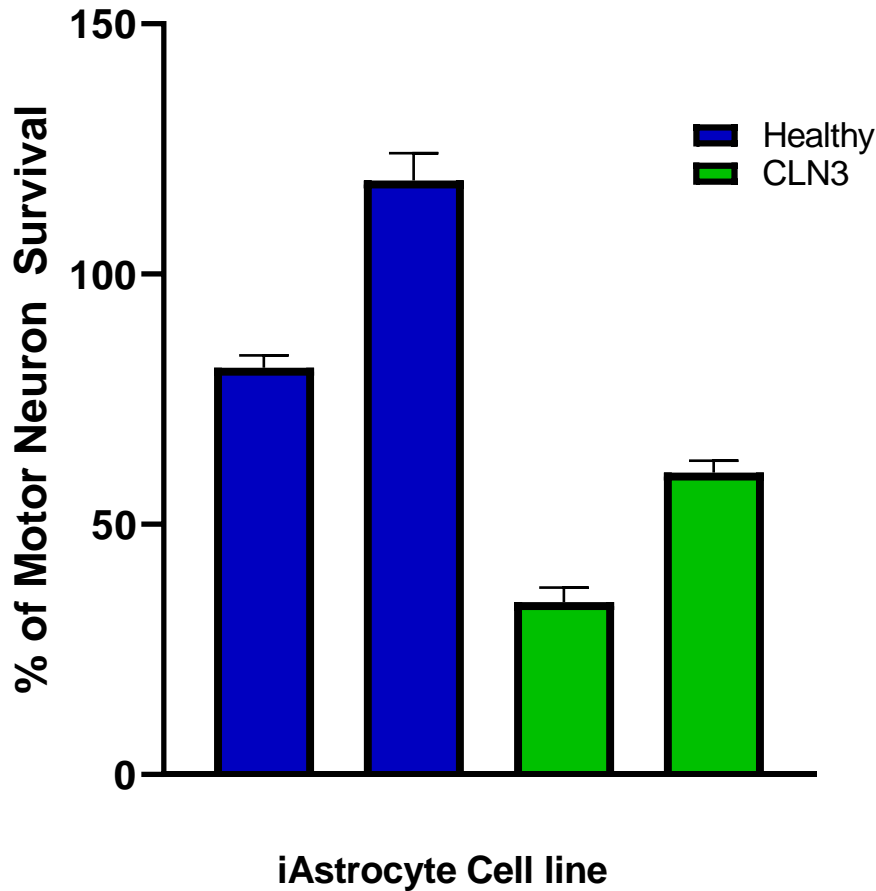
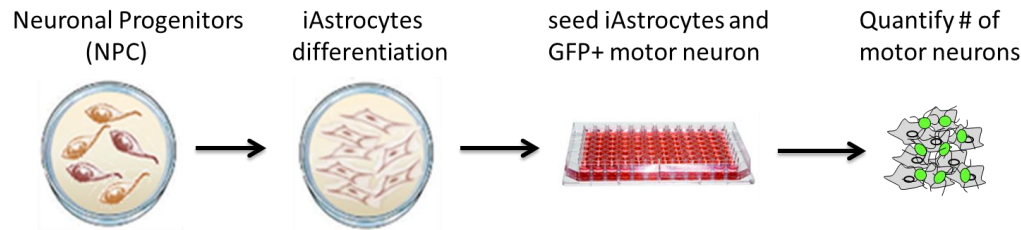
Juvenile batten disease (CLN3)



- **CLN3:** Neurodegenerative disorder:
Dysfunction of lysosomes:
Accumulation of storage material.
- Clinically:
 - Seizures
 - Progressive deterioration of cognition (dementia)
 - Motor function impairment
 - Rapid vision loss
 - Affects other systems (CV).



CLN3 patient Astrocytes are toxic to neurons



Healthy

CLN3

Rett Syndrome

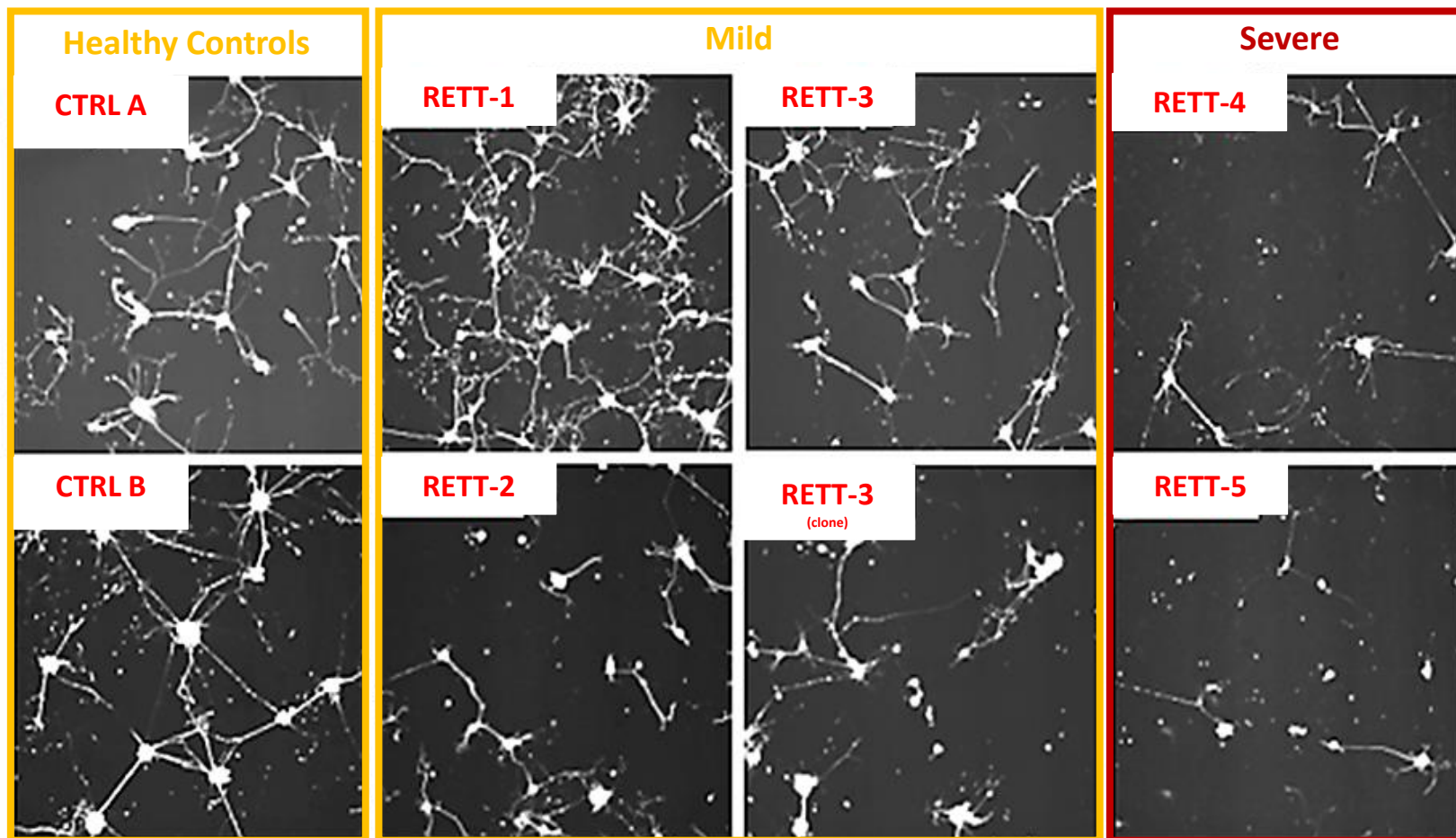
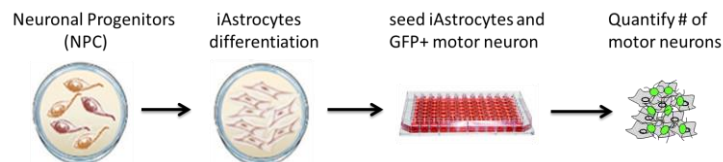


- X-linked neurodevelopmental Autism Spectrum Disorder (ASD)
- Clinically:
 - Loss of motor function
 - Loss of speech
 - Stereotypic hand gestures.
 - Seizures



Cellular Phenotype: global compaction of neurons with shortened/fewer neurites

Astrocytes from Rett patients show various severity towards motor neuron morphology and survival



SCN2A mutations

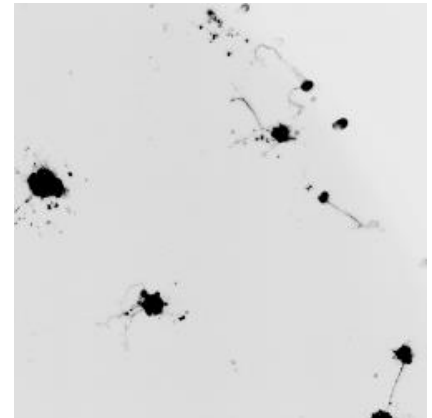
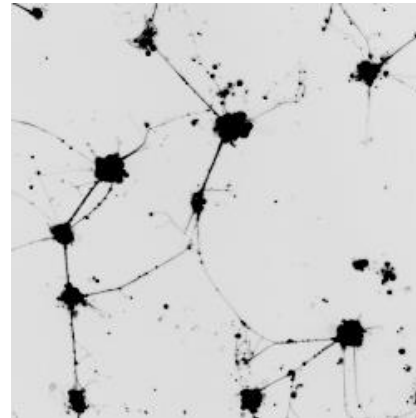
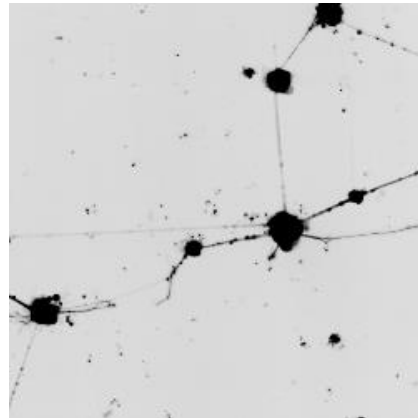
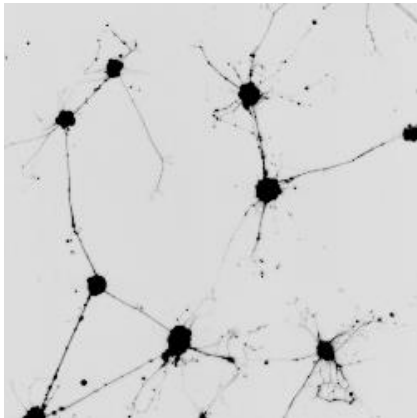
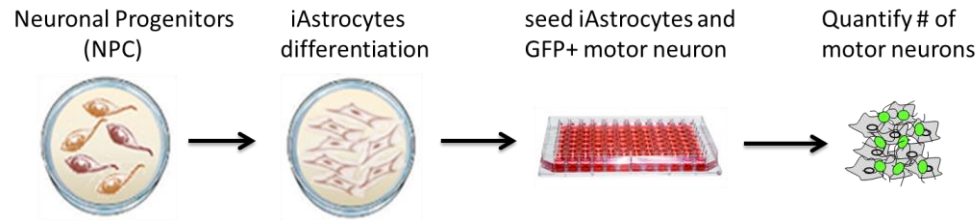


- SCN2A: Sodium channel → Helps propagate the electrical signals that neurons use to communicate
- Primarily expressed in excitatory neurons: Also in astrocytes.
- Neurodevelopmental disorders:
 - Epilepsy
 - Nonsyndromic intellectual disability
 - Autism spectrum disorders.



<https://www.scn2a.org/personal.html>

SCN2A Astrocyte toxicity



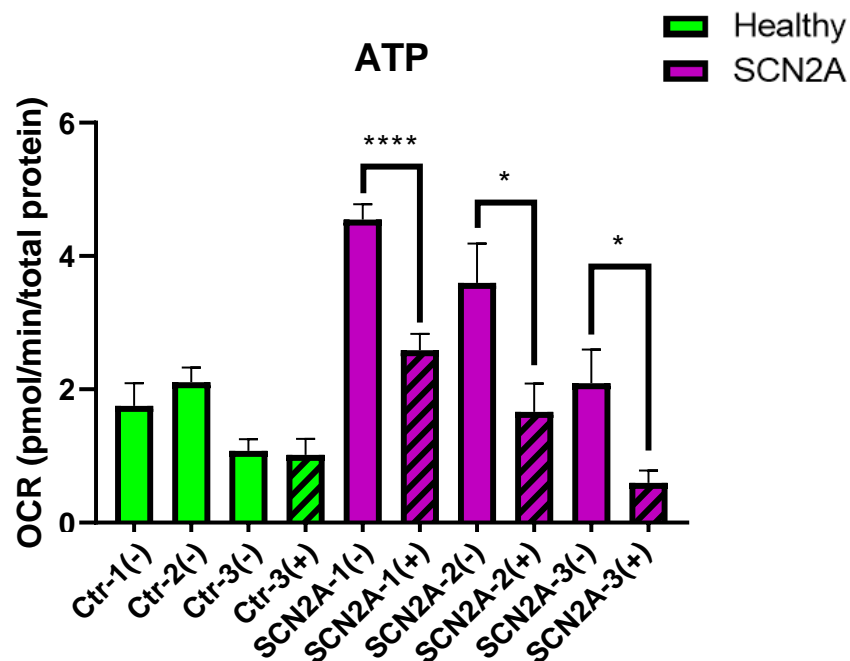
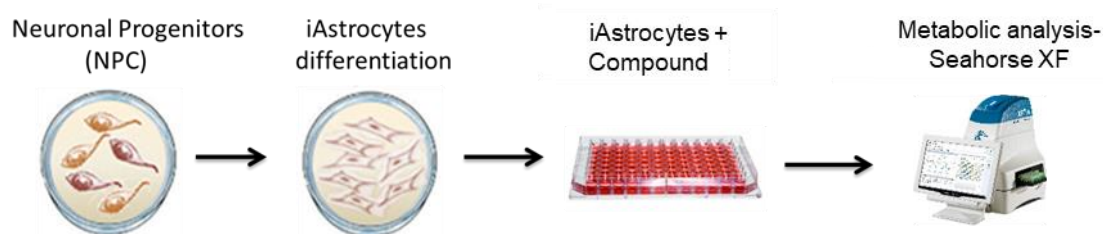
Healthy

SCN2A lines

NOT all SCN2A astrocytes are toxic to motor neurons.

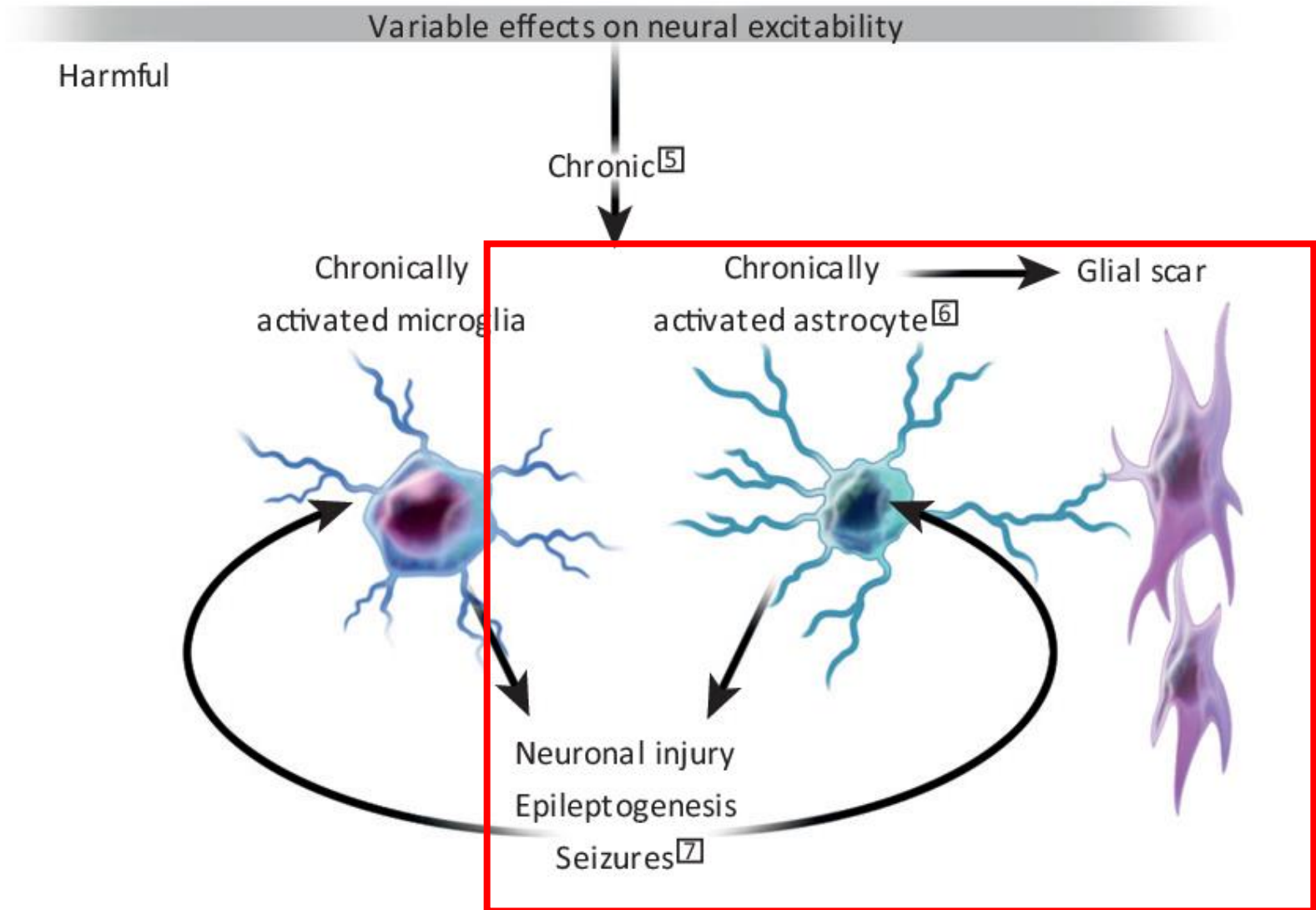


SCN2A patient astrocytes show alterations in mitochondrial function that can be reversed by compound treatment

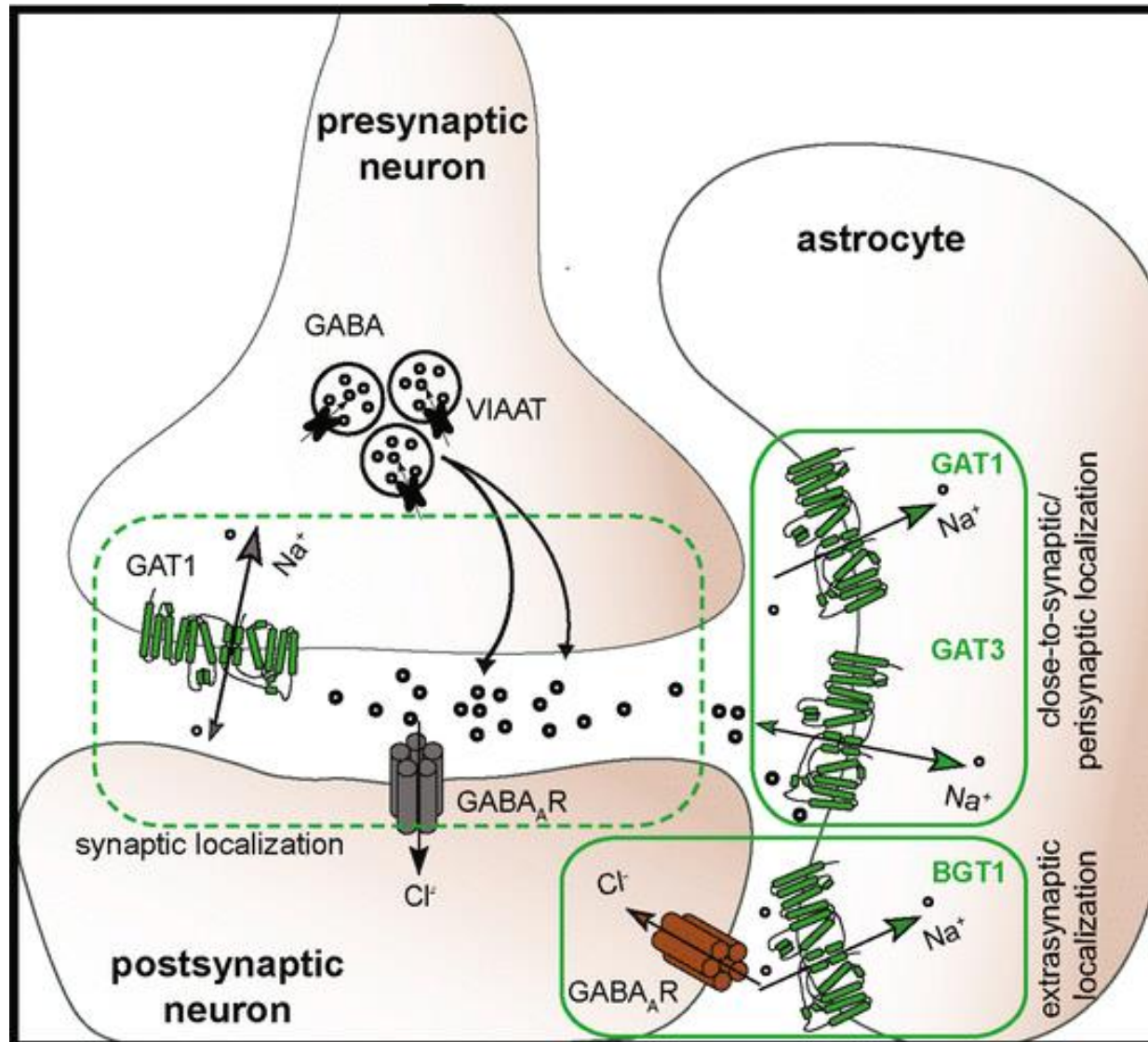


Pharmacological treatment restores mitochondrial activity of SCN2A astrocytes.

Glia contributes to the pathophysiology of epilepsy



GAT1 is expressed by presynaptic neurons and Astrocytes



Take home message



- Direct conversion is a promising tool to generate cell types of the CNS
- These cells can be used to study disease mechanisms, analyze commonalities and differences between individual patient subpopulations in different disease mechanisms.
- Useful tool to test potential new targets on a broad variety of backgrounds
- Could be a valuable tool to study SLC6A1

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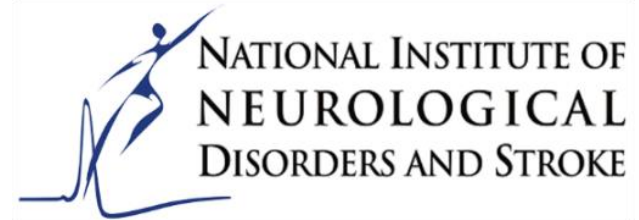
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FIGHT BATTEN DISEASE
FOUNDATION



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