Ceroid-lipofuscinosis, neuronal 3 (CLN3), Batten disease

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Project group

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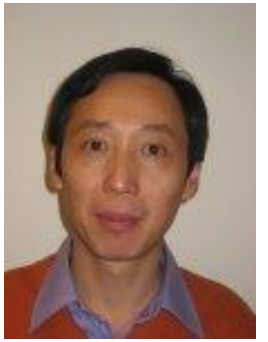


Mingyi Yang, PhD
Sen researcher, OUS

Outline

- Background (specially the impact of lysosmes and mitochondria)
- CLN3 projects - Bjørås group
  1. Modelsystems to study brain disease - mini brains/organoids
  2. What is the function of CLN3?
  3. Treatment strategies CLN3
  4. Biomarkers CLN3

Batten disease

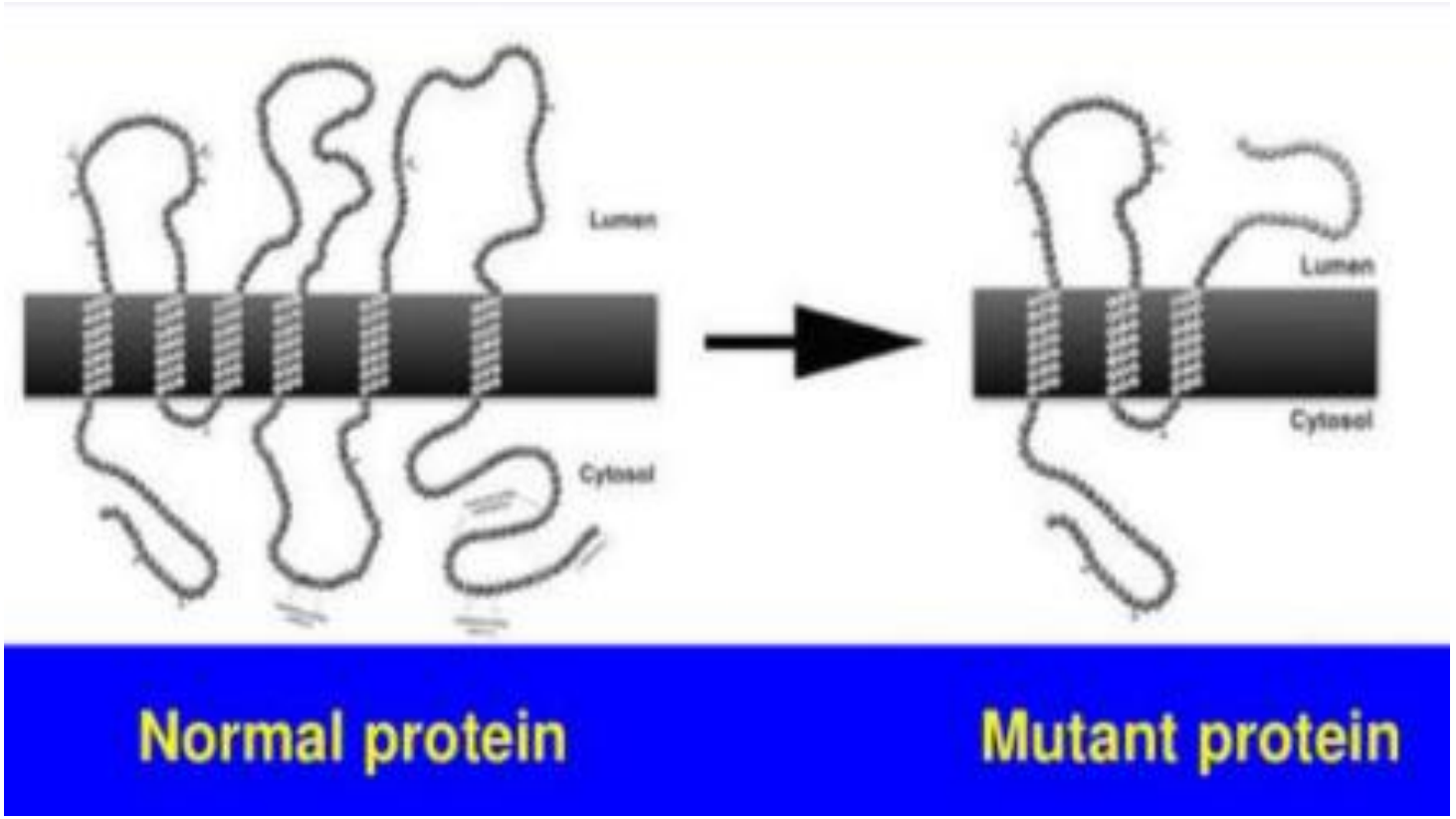
Lysosomal storage disorders (LSDs), caused by mutations in at least one of 13 genes

Most common form:
Ceroid-lipofuscinosis, neuronal 3 (CLN3), Batten disease

- Most frequent neurodegenerative disorders in children
- Globally, the prevalence is 1 in 100,000 births
- Genetic disease
- Incurable

Ceroid-lipofuscinosis, neuronal 3 (CLN3), Batten disease

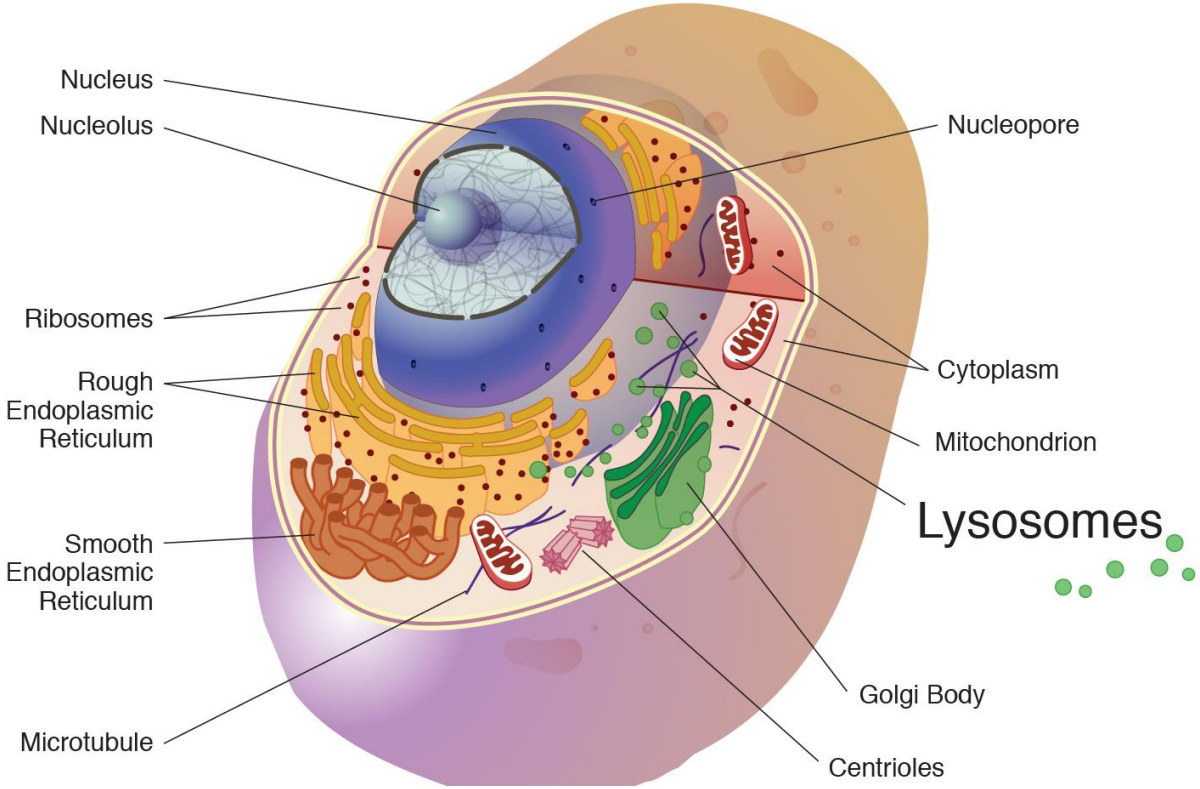
In 85 % of cases: caused by complete deletion of exons 7 and 8 in CLN3



Ubiquitously expressed protein in lysosomal membrane

Unknown function…

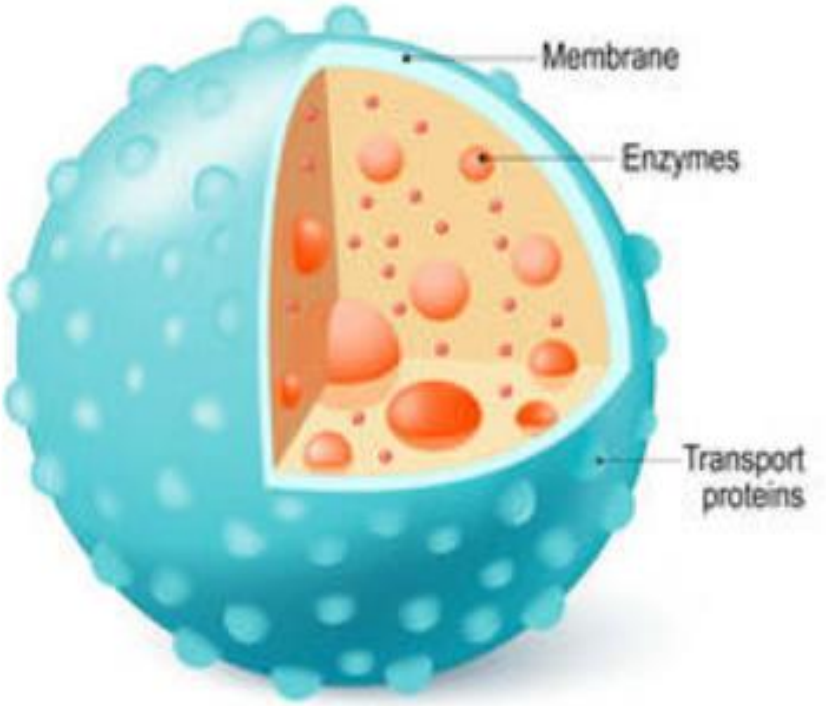
Lysosome is the center for cellular waste disposal:
degradation and recycling of cellular material



Other Functions:

- cellular nutrient sensing
- cell homeostasis
- response to infection
- energy metabolism

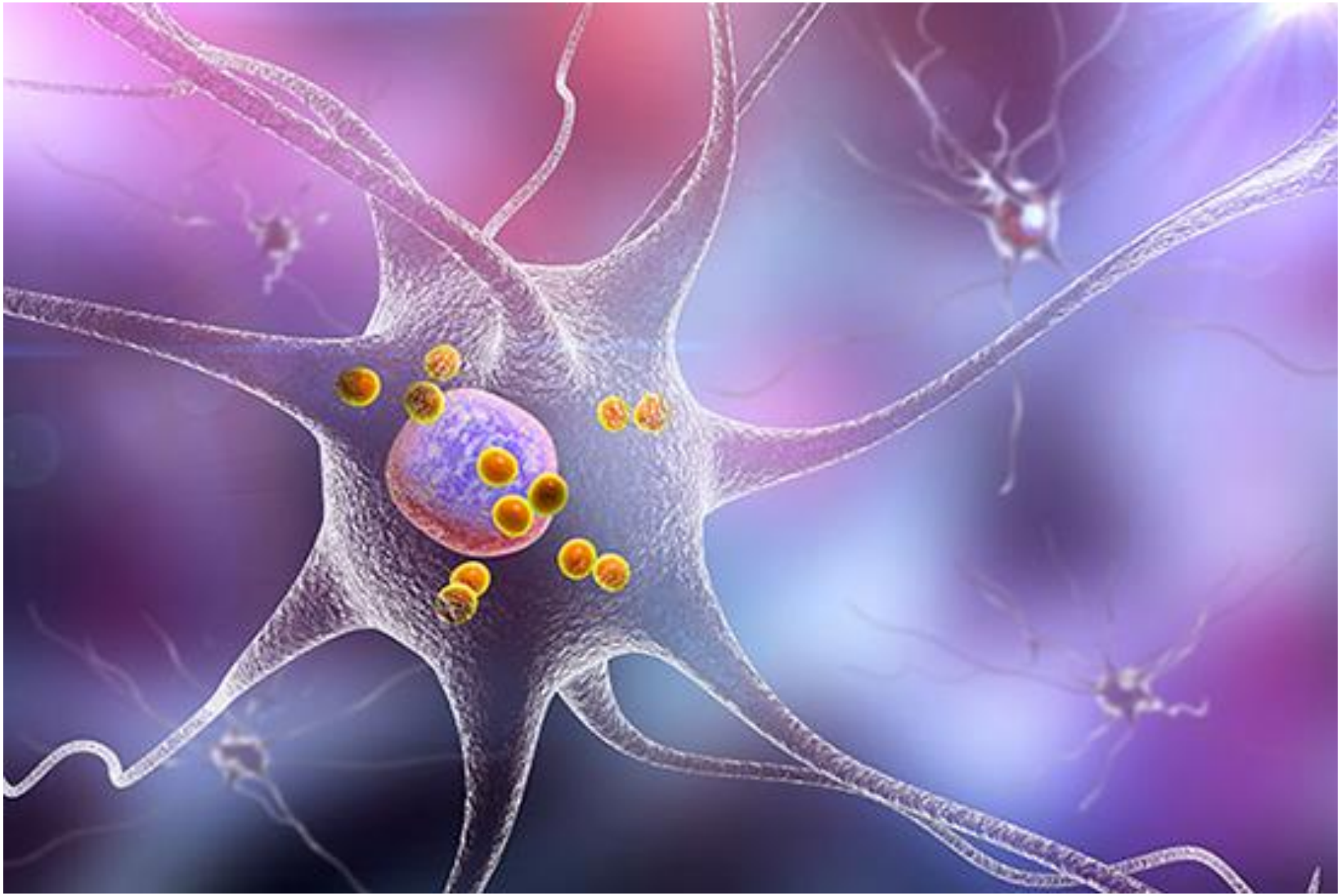
Lysosome



Defects in lysosomal functions can lead to:

accumulation of undigested or partially digested macromolecules (proteins) in lysosomes ('storage') → *Cell Damage*

CLN3 dysfunction is characterized by accumulation of waste in cells



...which leads to intellectual impairment and cognitive disorders

How???

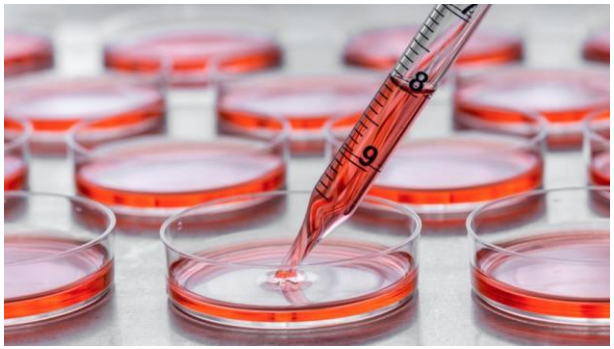
HOW???

We need to understand the pathology at organ, cell and molecule levels

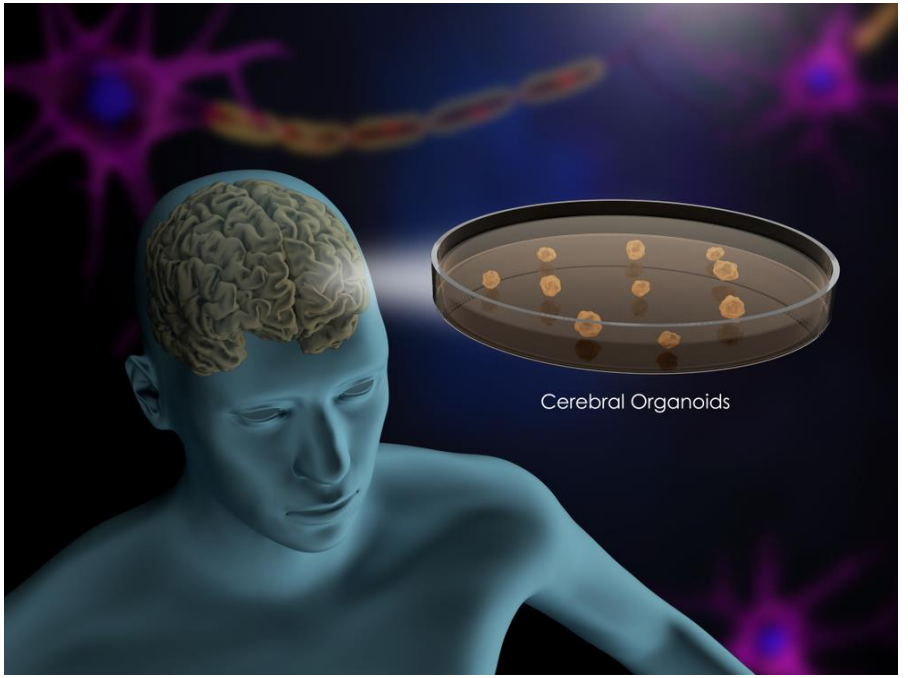
↓

Important for improvement and acceleration of the drug development field

Major challenge:
Paucity of appropriate preclinical models

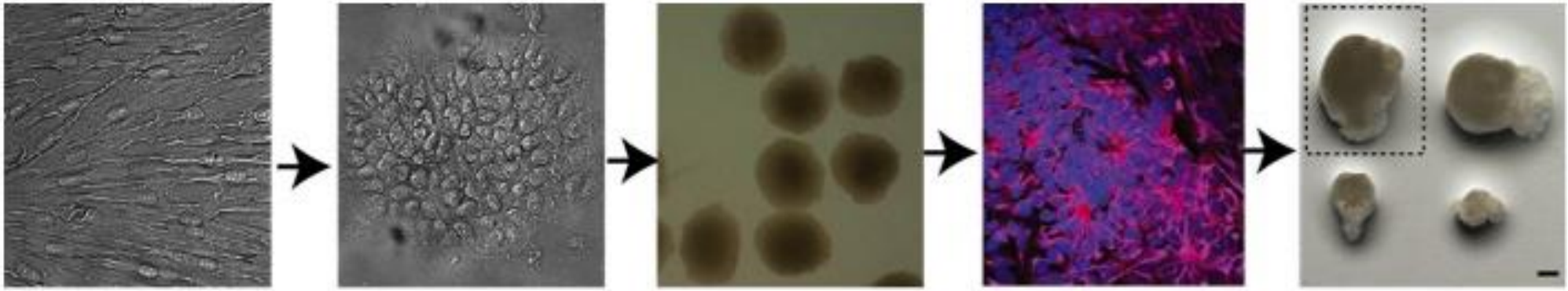


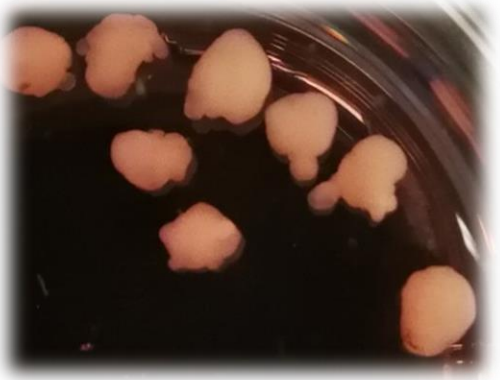
Mini-brains (brain organoids)



- Generated from cells obtained from skin biopsies
- Good preclinical models
- Great promise for personalized treatment
- Enable accurate prediction of drug responses or outcomes after therapeutic interventions in patients

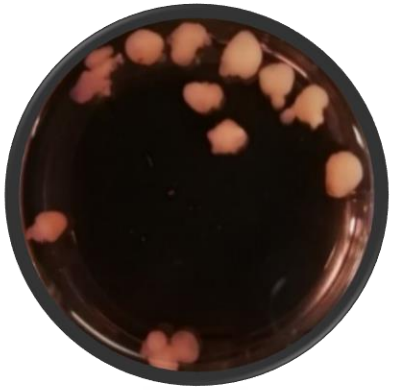
Generation of brain organoids from a skin biobsy



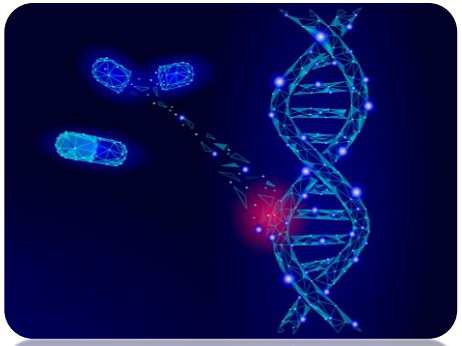


Brain organoids as a precision medicine approach to identify new prognostic markers and treatment modalities for CLN3 Batten disease

Aims of the Project



- *Generate brain organoids (whole brain and specific brain regions)*
- *Recapitulate disease progression*
- *Explore new treatment strategies based on gene therapy*

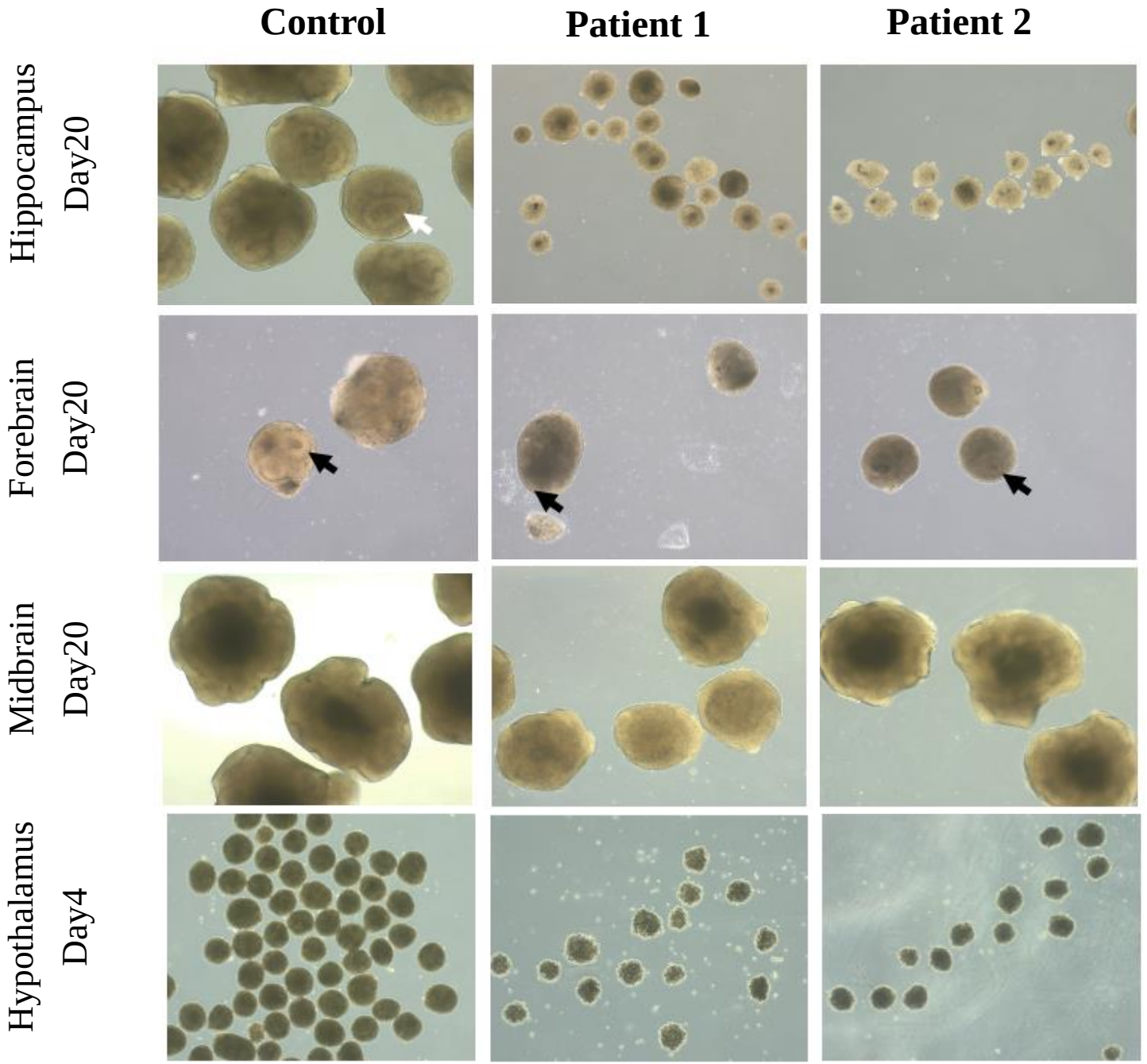


Long-term goals

- *Implement our gene therapy strategy in clinical trials*
- *Identify new diagnostic and prognostic markers*
- *Identify new therapeutic targets for Batten disease*

Characterization of pathomechanisms

Whole brain and Region-Specific Brain Organoids as Preclinical Models

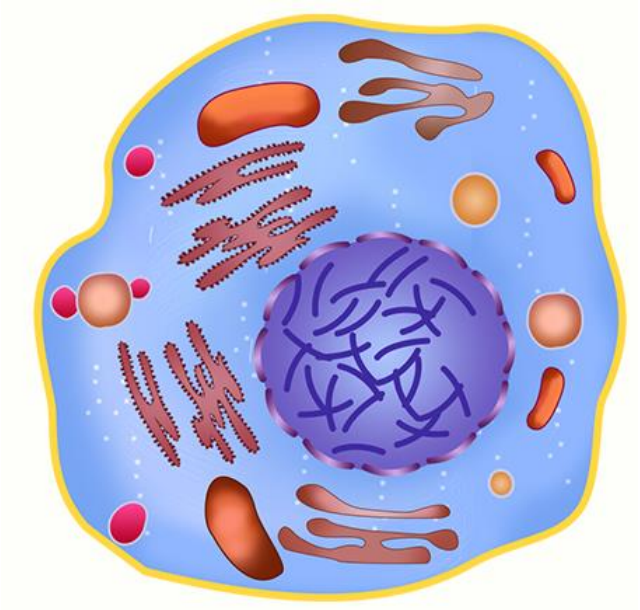


Xiaolin Lin

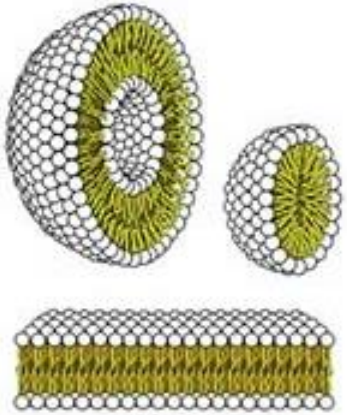
Ingrid Åmellem

Representative bright-field images.
White arrow: subgranular zone (SGZ). Black arrows: ventricular-subventricular zone (VZ/SVZ).

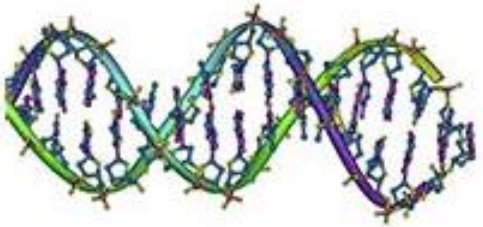
Human Cells



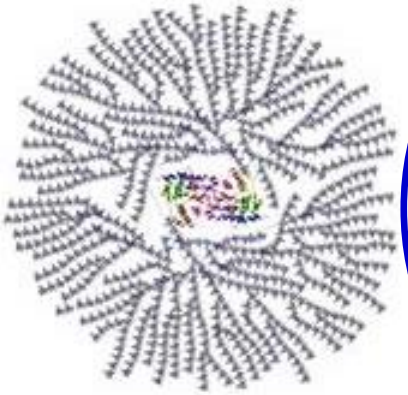
Biomolecules



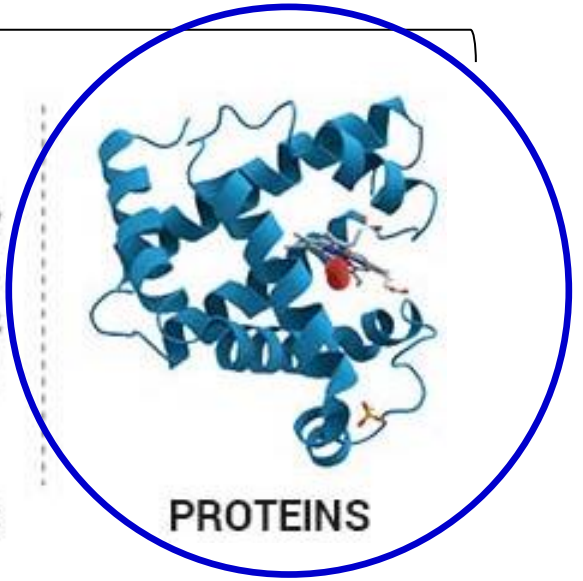
LIPIDS



NUCLEIC ACIDS

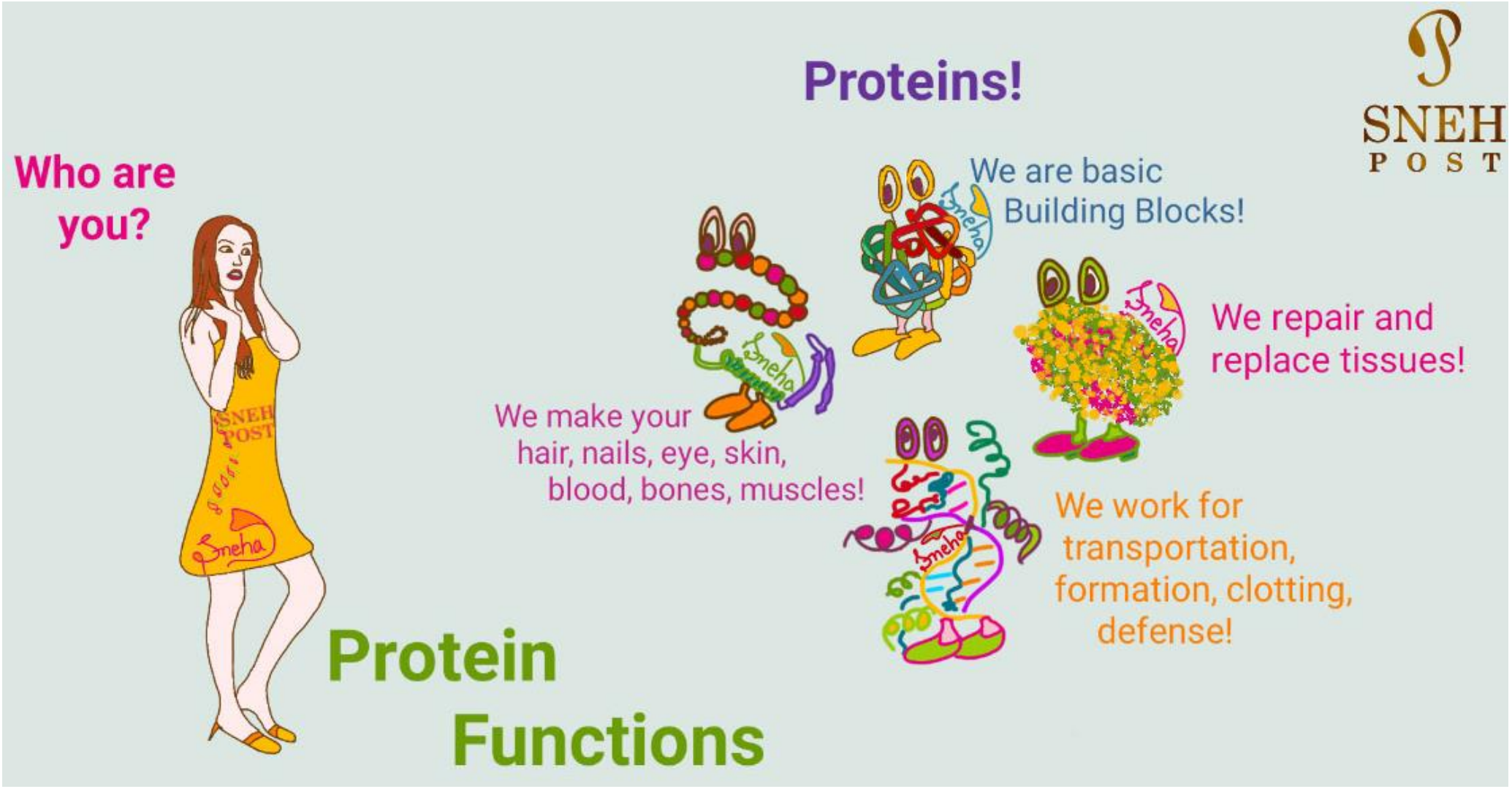


CARBOHYDRATES



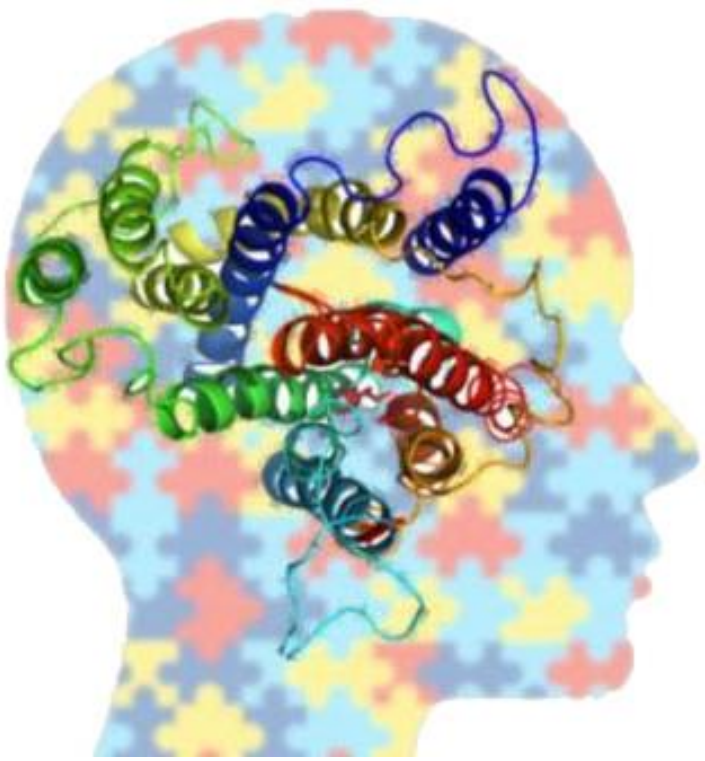
PROTEINS

Protein carry out the majority of functions within the cell



https://www.snehpost.com/2015/03/protein-function.html

Compare protein profiles of healthy controls and patients' mini-brains



Investigated more than
6000 proteins in mini brains

Most important finding:

Mitochondria dysfunction

Mirta ML Sousa

Mingyi Yang

Analysis of proteins and processes affected by CLN3 dysfunction may reveal:

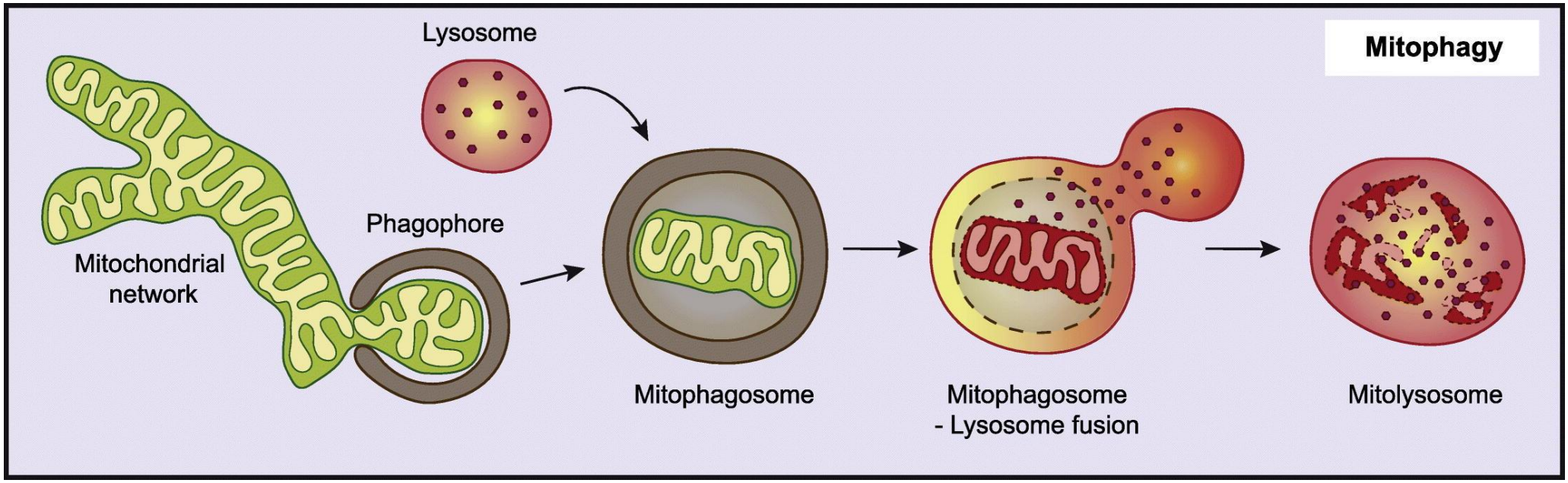
- *novel diagnostic and prognostic biomarkers*
- *novel therapeutic targets for CLN3 Batten disease*

Mitochondrial dysfunction in brain

- can decrease fatty acid metabolism
- important factor associated with neurological diseases

How can the cell remove aged or dysfunctional mitochondria?

Mitophagy



https://www.sciencedirect.com/science/article/pii/S0022283619304292#f0025

Removal of aged or dysfunctional mitochondria (ongoing studies)

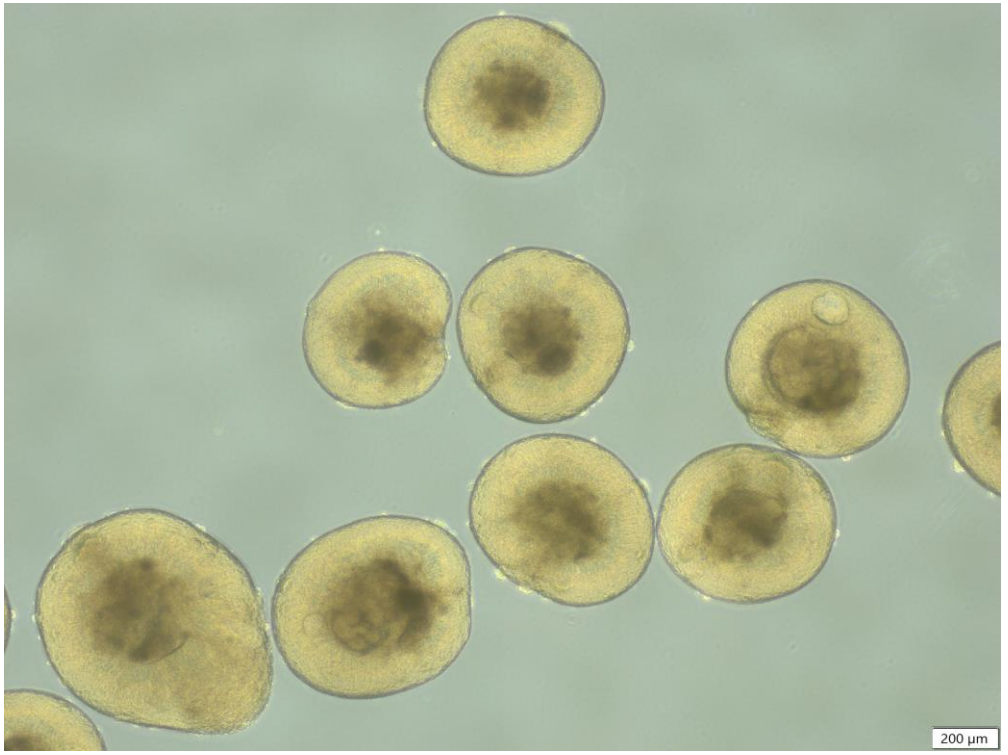
Generation of eye organoids

Blindness is a burden for children with Batten disease

Jørn-Ove Schjølberg

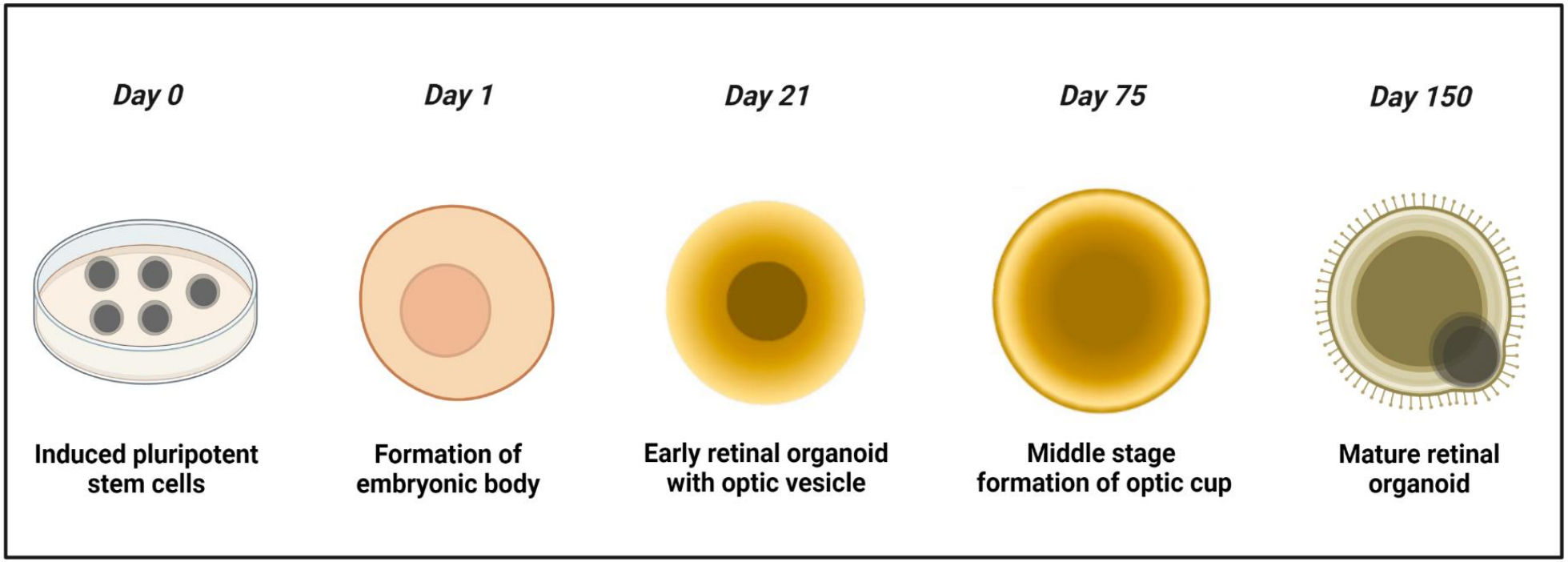
Borghild Farsund

Retinal organoids



Retinitis pigmentosa type 11 (RP11)

- We have established a protocol for generating iPSC-derived retinal organoids
- Model system to test treatments and study pathomechanisms in RP11
- Timeline: >150 days for mature retinal organoids
- Photoreceptor maturation takes time
- Working on reducing this timeframe



Implement the protocol to:

- *study mechanisms related to blidness in CLN3 eye organoids*
- *explore treatment strategies (gene therapy)*

Explore new treatment modalities

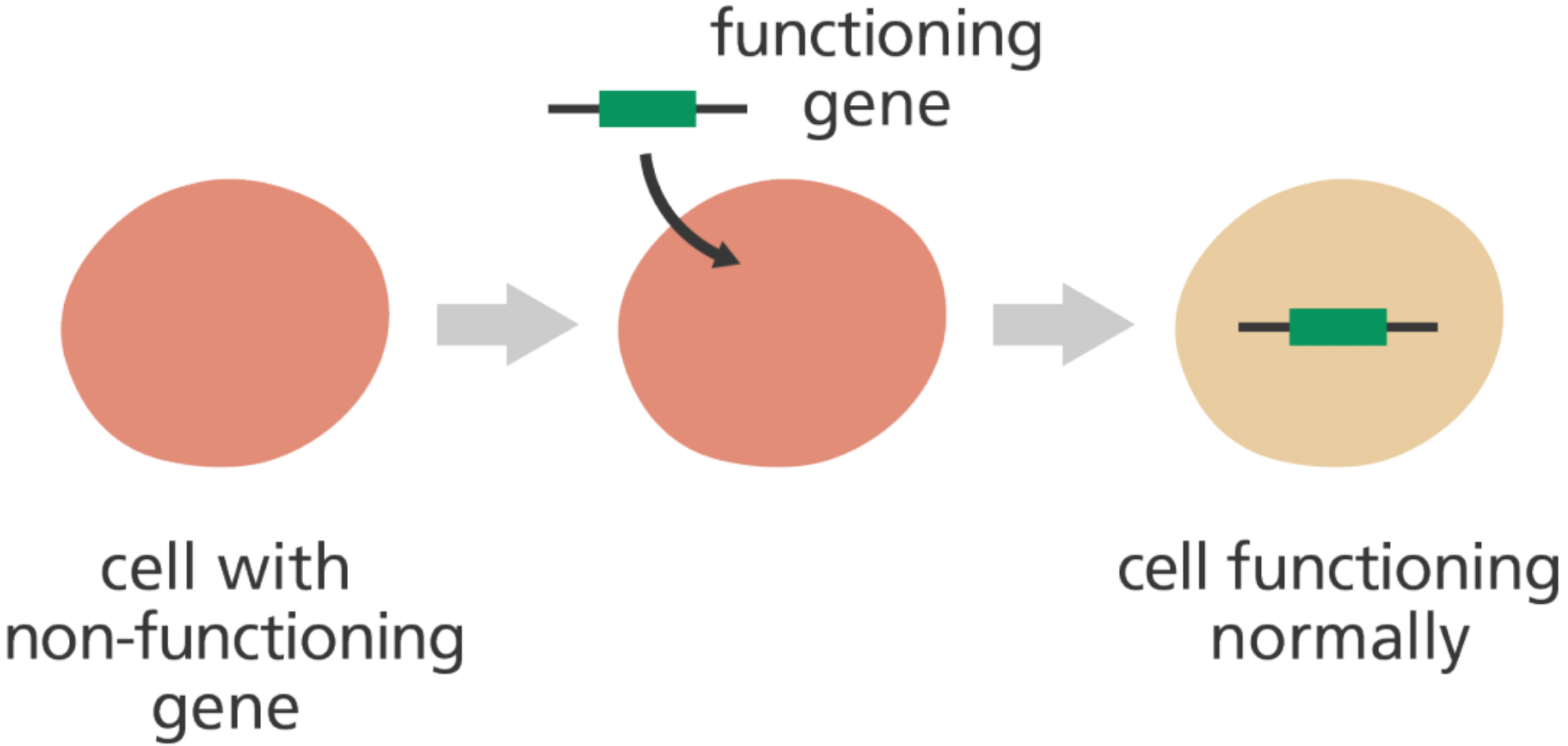
why Gene therapy ?

- No other treatment options with substancial improvement
- Severe debilitating disorders (benefit/risk ratio is high)
- Potential for life-changing long-lasting effect with single treatment

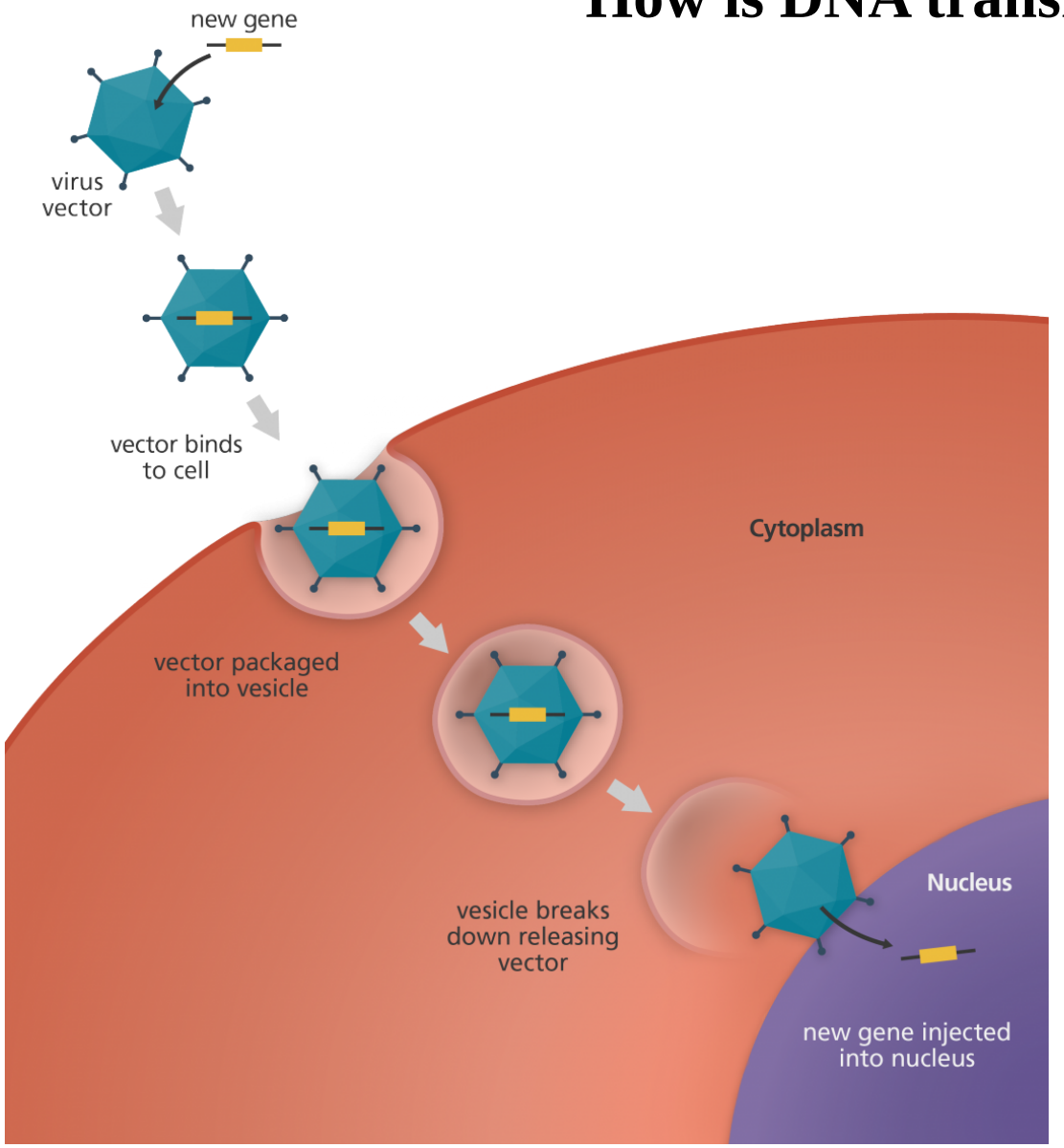
Challenges:

- Location/wide distribution of affected cells make treatment difficult
- Can trigger unfavorable immune response

Gene augmentation therapy



How is DNA transfer done?



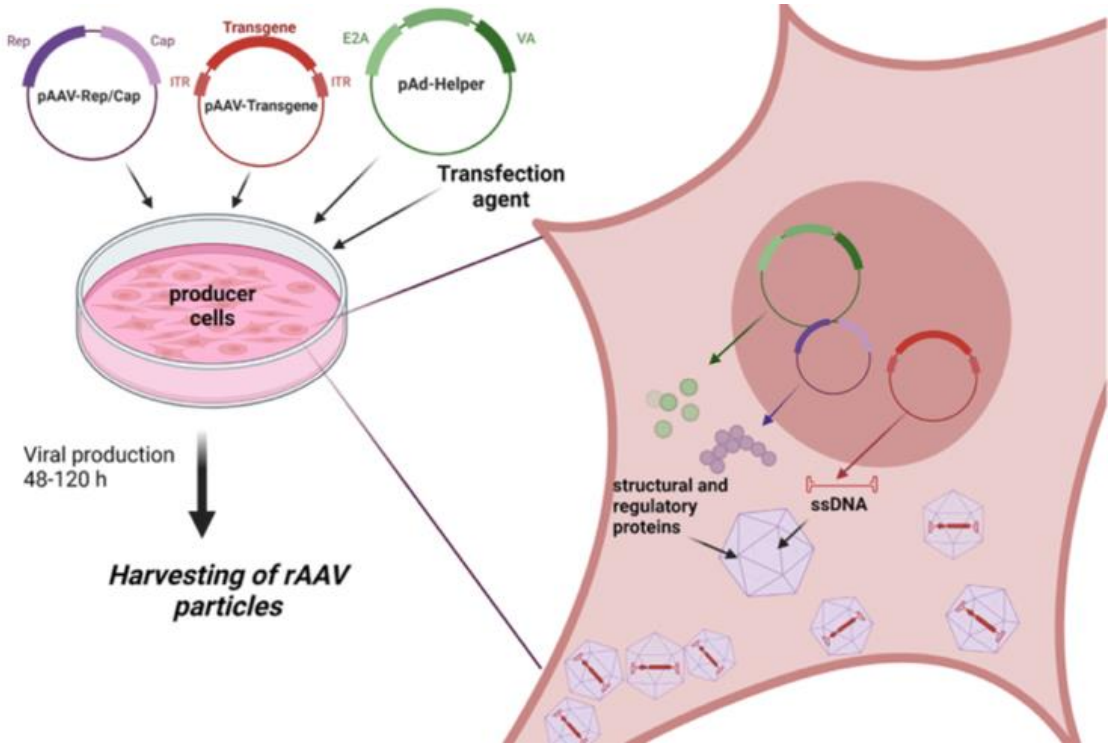
Implement the most successful gene therapy strategy in clinical trials

AAV-basert genterapi strategier for øye og hjerne

Hovedbestanddelene i en AAV vektor:

- **Promoter**; starter produksjon av gentranskript i cellen. I dette prosjektet prøver vi i første omgang ut tre ulike promotere
- **Gen**; i prinsippet kan man sette inn et hvilket som helst gen. I dette prosjektet tester vi ut CLN3 og antiinflamatoriske gener

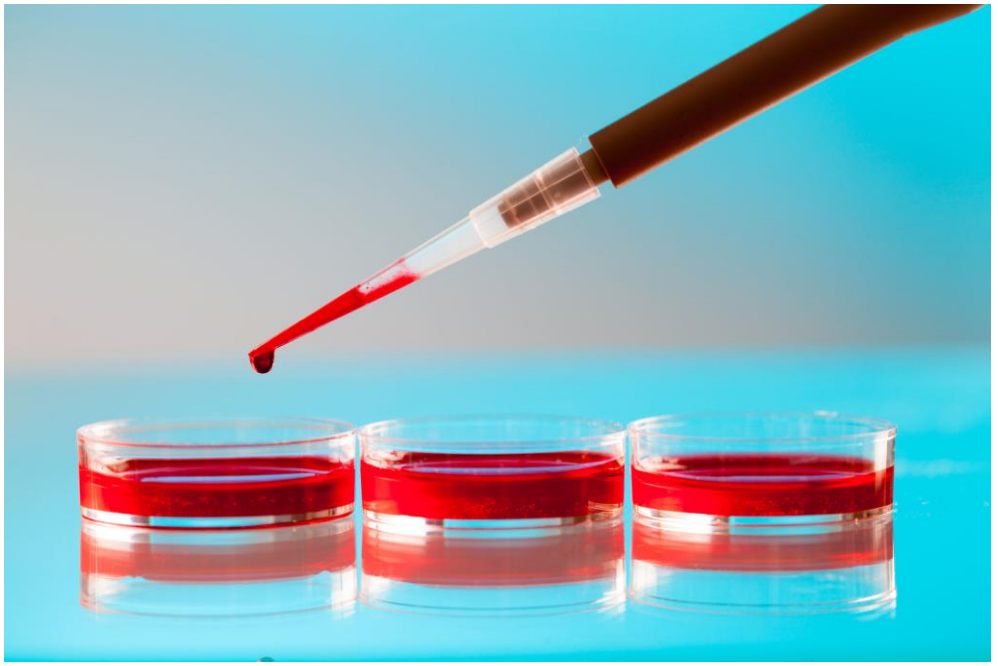
Produksjon av AAV ved hjelp av et trekomponent system



AAV basert genterapi status mai 2023

1. **Design av AAV vektorer**
2. **Konstruksjon av AAV vektorer**
3. **Produksjon av AAV vektorer (Mai 2023 -)**
4. **Produksjon av AAV partikler, liten skala (August 2023 -)**
5. Testing og karakterisering av AAV i cellemodeller, inkludert hjerne og retinale organoider
6. Testing og karakterisering i dyremodeller
7. Optimalisere produksjon av AAV for GMP manufacturing
8. GMP Manufacturing av AAV
9. Klinisk utprøving

Identification of Biomarkers in Blood



Importance of identification of biomarkers for:

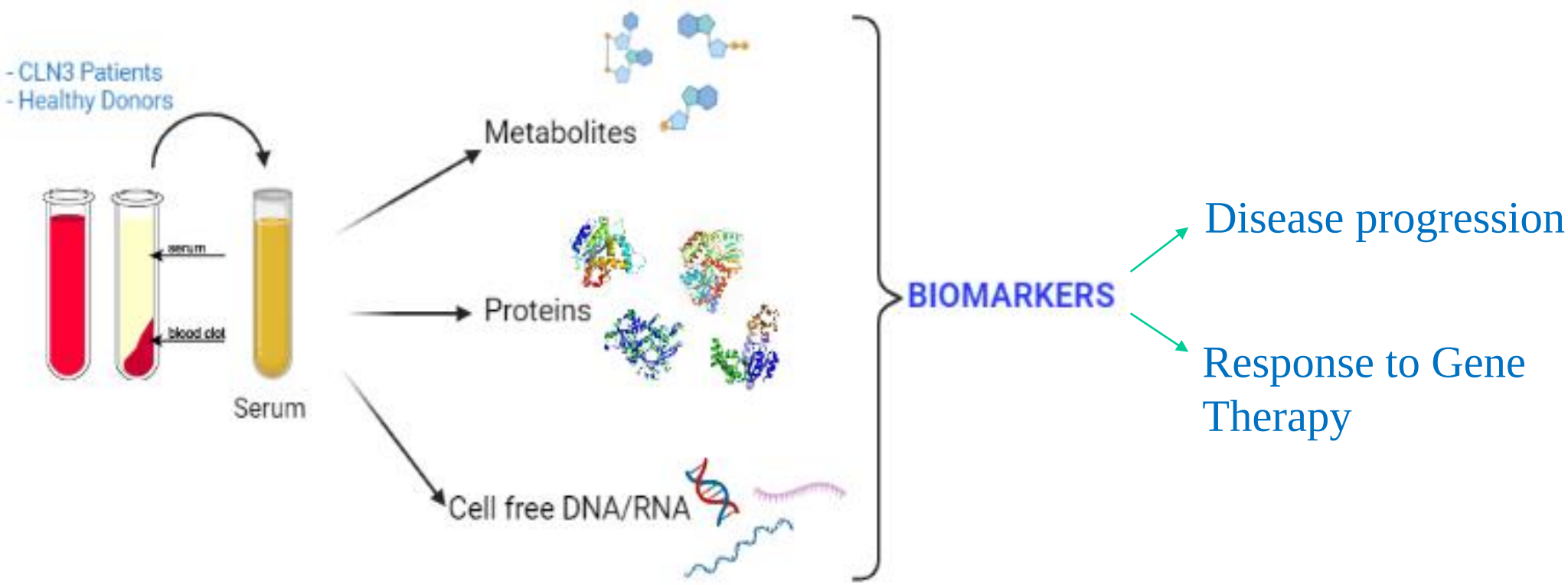
- early diagnosis of batten disease
- response to therapy
- monitoring of disease progression

Biomarker project

Collect blood from:

(i) Healthy donors and CLN3 patients at different stages of the course of disease

(ii) CLN3 patients before and after gene therapy



CLN3 prosjekt

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Wannan Tang
Mingyi Yang
Jørn-Ove Schjølberg

Ingrid Helland, OUS

Rune Østern, St.Olavs Hospital

Prof. Michael R Volkert
University of Massachusetts Medical School

Takk for støtte og samarbeid med foreldre og barn i NCL foreningen!!!

Laboratoriesenteret, NTNU, St Olav

Rikshospitalet