CLN2 Batten Disease

Internal Dossier

Tania Sultana, PhD

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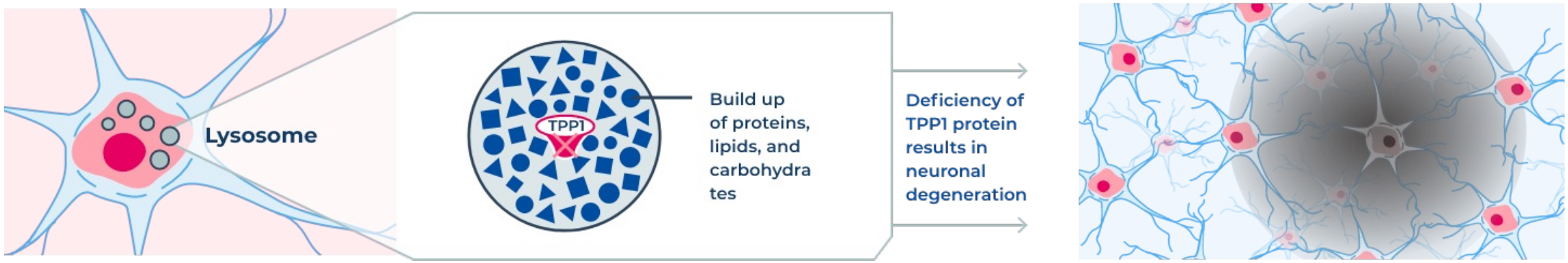
CLN2 Batten Disease Overview

CLN2 Batten Disease Overview

- Batten disease is the common name for a broad class of rare, fatal, heterogeneous group of **pediatric neurodegenerative disorders**, also known as Neuronal Ceroid Lipofuscinoses (**NCL**)[1].
- CLN2 Batten disease is one of the most common forms of NCL, commonly presents as the late–infantile phenotype.
- CLN2 Batten disease is caused by mutations in the **CLN2** gene, which encodes for tripeptidyl peptidase 1 (**TPP1**) protein.
- TPP1 breaks down proteins in the lysosomes of neurons[1]. Thus, CLN2 Batten disease is a lysosomal storage disorder (LSD).
- CLN2 mutations result in either reduced activity or inactivation of the TPP1, **accumulating ceroid lipofuscin in the lysosomes**, massive glial activation and neuronal loss.



CLN2 Batten Disease Overview

CLN2 disease presents both classically and atypically[2]:

- **Classic phenotype**:

Late–infantile onset, progress in a rapid and predictable manner, and present most cases. Also referred to as late-infantile neuronal ceroid lipofuscinosis **(LINCL)** (OMIM # 204500).

- **Atypical phenotype:**

Residual TPP1 enzyme activity is more common.

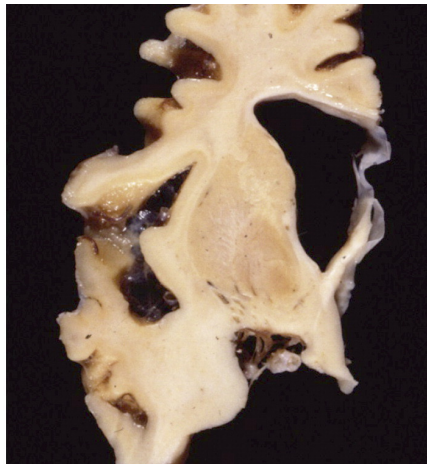
Age of onset, rate of progression, and symptom progression vary:

- Infantile, with onset under the first year
- Late–infantile onset with a protracted progression and patients may live into their 20s
- Juvenile onset with a classic progression

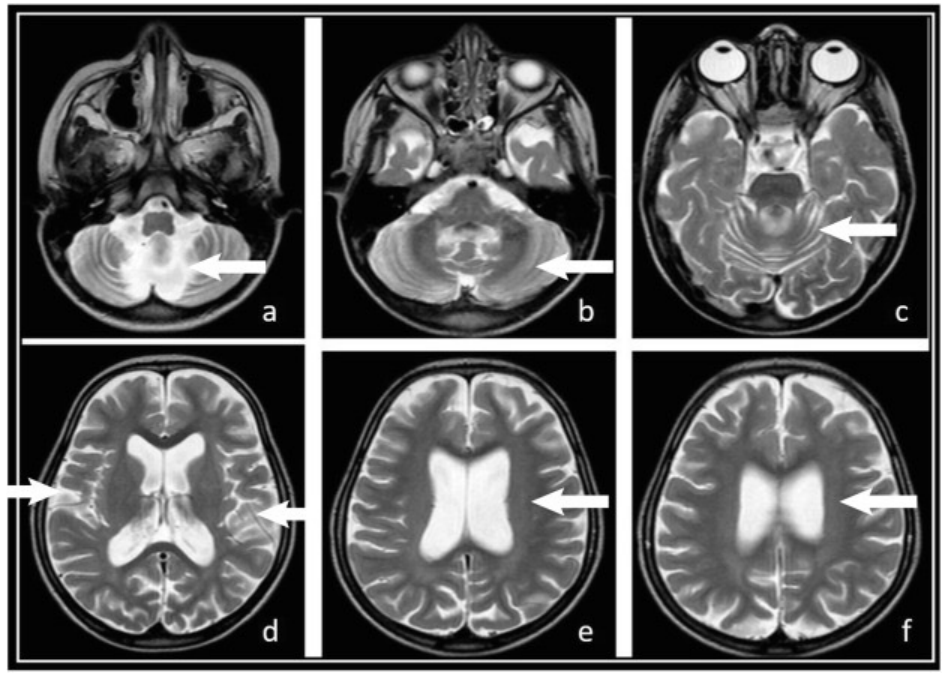
CLN2 Batten Disease Neuropathology

Neuropathology of CLN2 Batten Disease

- The molecular consequences of CLN2 mutations lead to cell dysfunction and ultimately death.
- Brain weight drops to 500–700g due to cerebral cortical atrophy accompanied by a reduction in white matter, and enlargement of: cerebrospinal fluid-carrying spaces, the subarachnoid space, and the ventricles[1,2].
- Subtle MRI features of atrophy can support early diagnosis[3].
- Neuron loss for small neurons of layers III and V are found under light microscope[1].



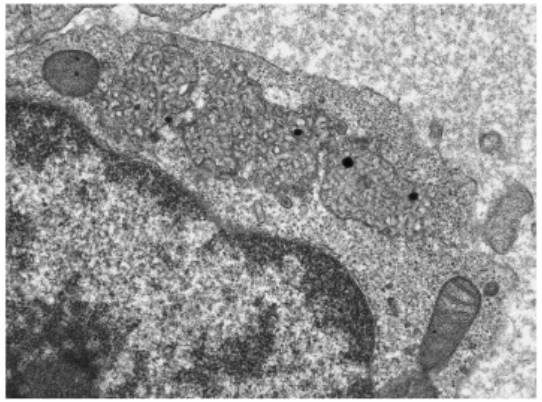
Atrophic cerebral cortex



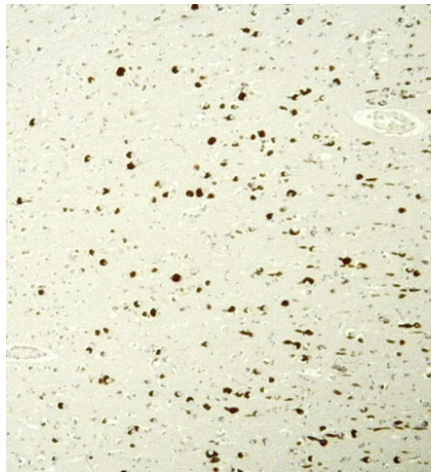
T2-weighted axial sections from an MRI scan taken 1 year after the onset of symptoms. This scan demonstrates mild to moderate cerebellar (a, b, c) and cerebral (d) atrophy, and linear hyperintensity of central white matter (e, f).

Neuropathology of CLN2 Batten Disease

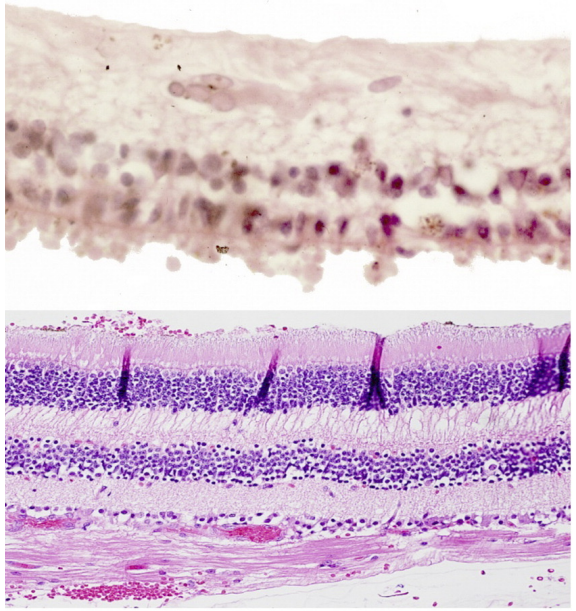
- Classic CLN2 disease shows curvilinear profiles*,[1].
- Lipopigment formation is a hallmark of NCL diseases. SCMAS**, a component of the lipopigments are found by immunohistochemistry and electron microscopy[1].
- Atrophy of the retina is less pronounced compared to other NCLs.



Curvilinear profiles with fragments of dense deposits



Nerve cells with SCMAS-containing lipopigements



Top: Atrophic retina with only remnants of photoreceptor inner segments and severe loss of neurons

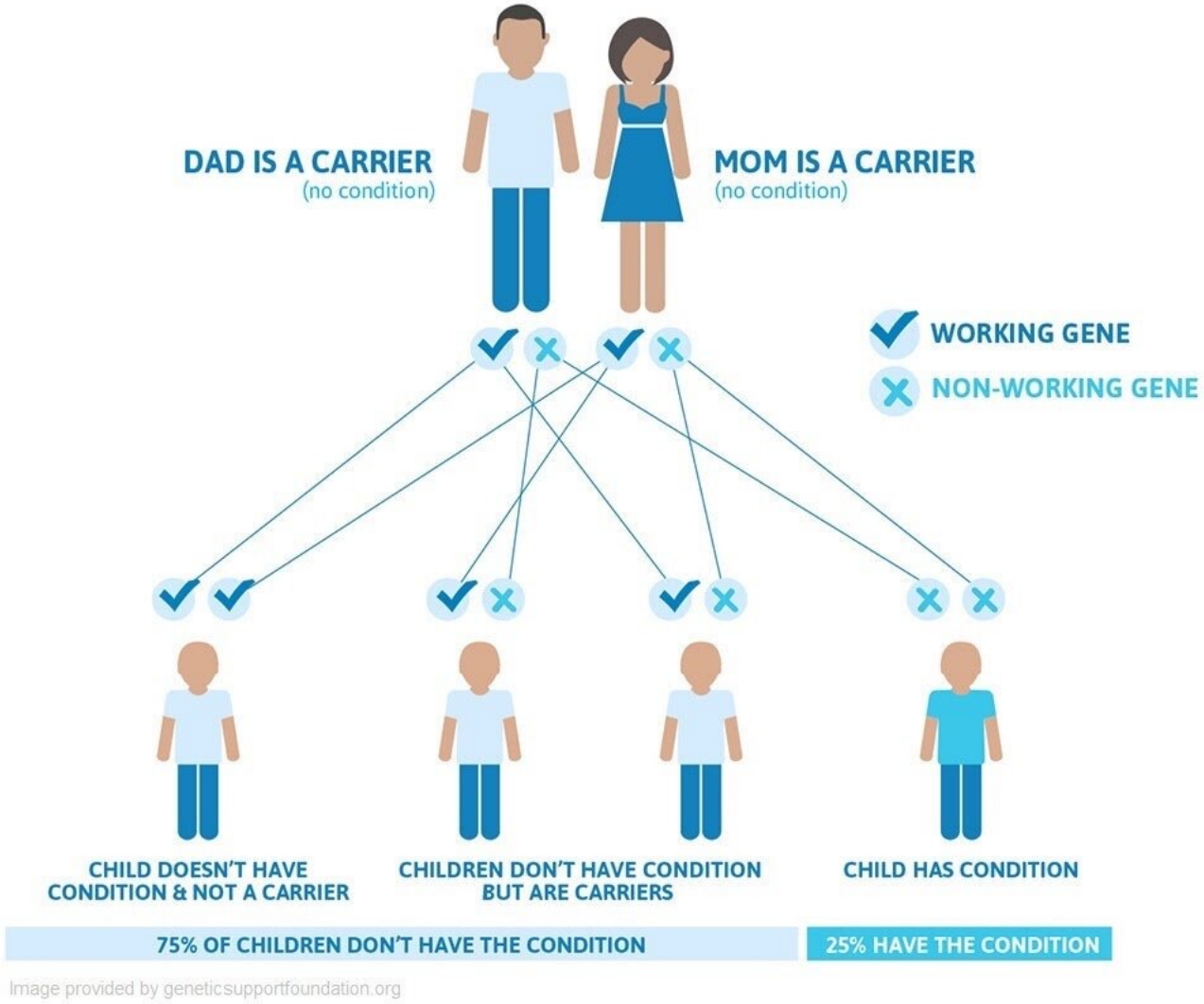
Bottom: Normal retina

CLN2 Batten Disease Epidemiology

CLN2 Batten Disease is an Inherited Disease

- The disease-causing genetic mutation in CLN2 gene is **autosomal recessive**, located on chromosome 11p15.
- When both parents carry one copy of the defective CLN2 gene, there is **25%** probability of having an affected child.

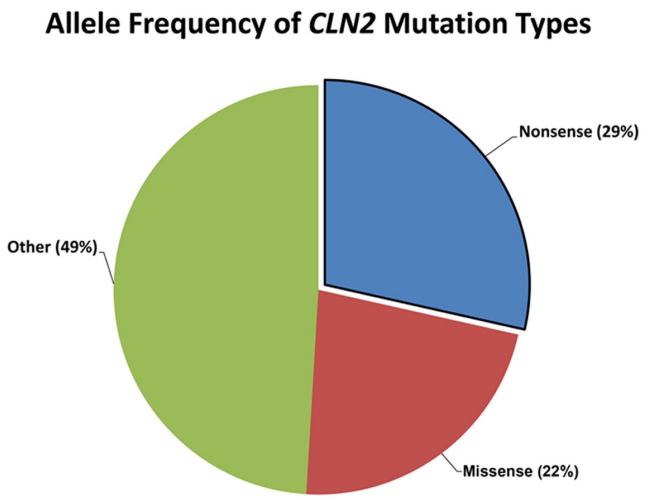
Probability	Children Inherit:
One-in-four (25%)	**Affected:** Two copies of the defective CLN2 gene.
One-in-four (25%)	**Unaffected:** No copies of the defective CLN2 gene.
One-in-two (50%)	**Carrier but unaffected:** One copy of the defective CLN2 gene and one copy of the healthy gene.



CLN2 Batten Disease is an Inherited Disease

- CLN2 mutations include splice-junction mutations, missense mutations, nonsense mutations, small deletions, and single-nucleotide insertions.
- Missense and nonsense mutations account for >50% of the mutation allele frequencies[1]; of which missense mutations contribute 22% and nonsense mutations contribute 29% (Fig A).
- The most common CLN2 disease mutations are (Fig B):
  - Intronic transversion c.509-1G>C that results in **altered transcript splicing**.
  - Exonic transition c.622C>T that results in the **p.R208X nonsense mutation**.

A



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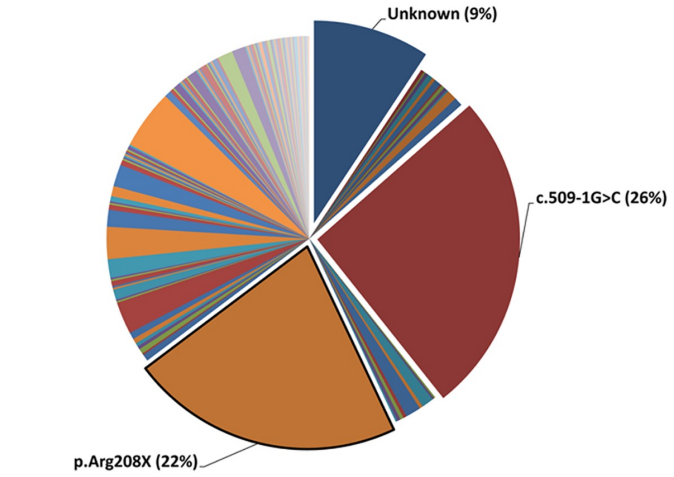
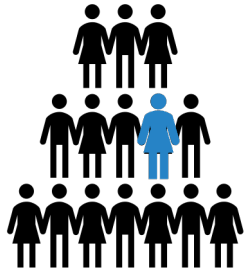


Global Prevalence

- Batten disease prevalence including the CLN2 disease is poorly reported[1].
- There are large geographical variation[1-5].

All Batten Diseases[1,5]

- Batten disease affects **~3 of every 100,000 babies** in the United States.
- People of Scandinavian or Northern European descent are more likely to have the disorder.
- In Northern Europe, **~1 of every 25,000 babies** is born with Batten disease.
- **Highest** incidence in **Newfoundland**: ~14 per 100,000 babies[2].
- **Lowest** incidence in **Italy**: ~0.6 per 100,000 babies[3].



CLN2 Batten Disease

- >300 cases worldwide have been reported.
- Affects **less than 1 in 100,000 live births worldwide**[1].
- ~50% of affected South American babies show slow disease progression[6].
- **Highest** incidence in **Newfoundland**: ~9 per 100,000 live births[2].
- **Lowest** incidence in **Portugal**: ~1.5 per 100,000 live births[3].

CLN2 Batten Disease Symptoms

18 months

Age 3

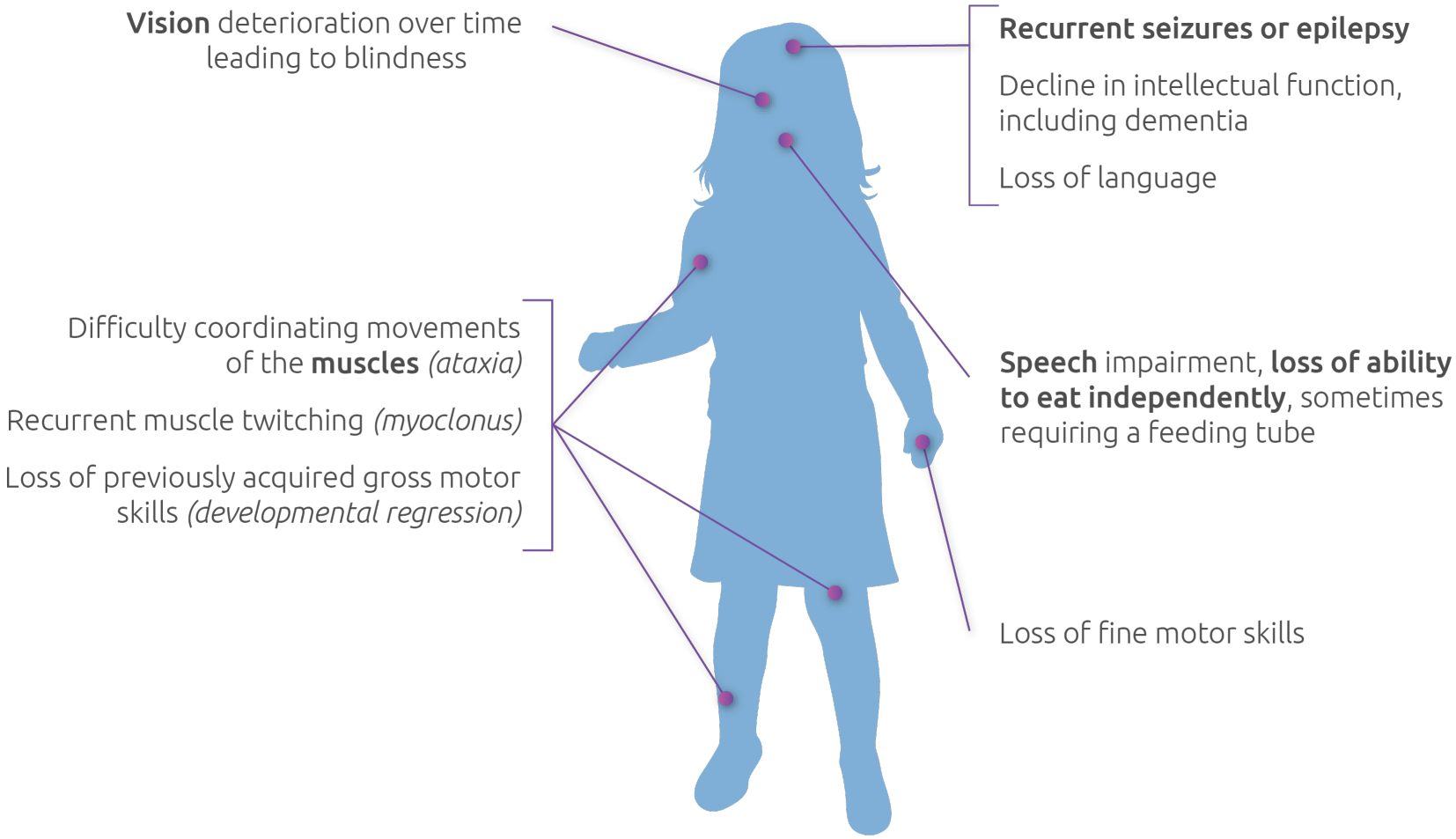
Age 5

Age 7

Age 10

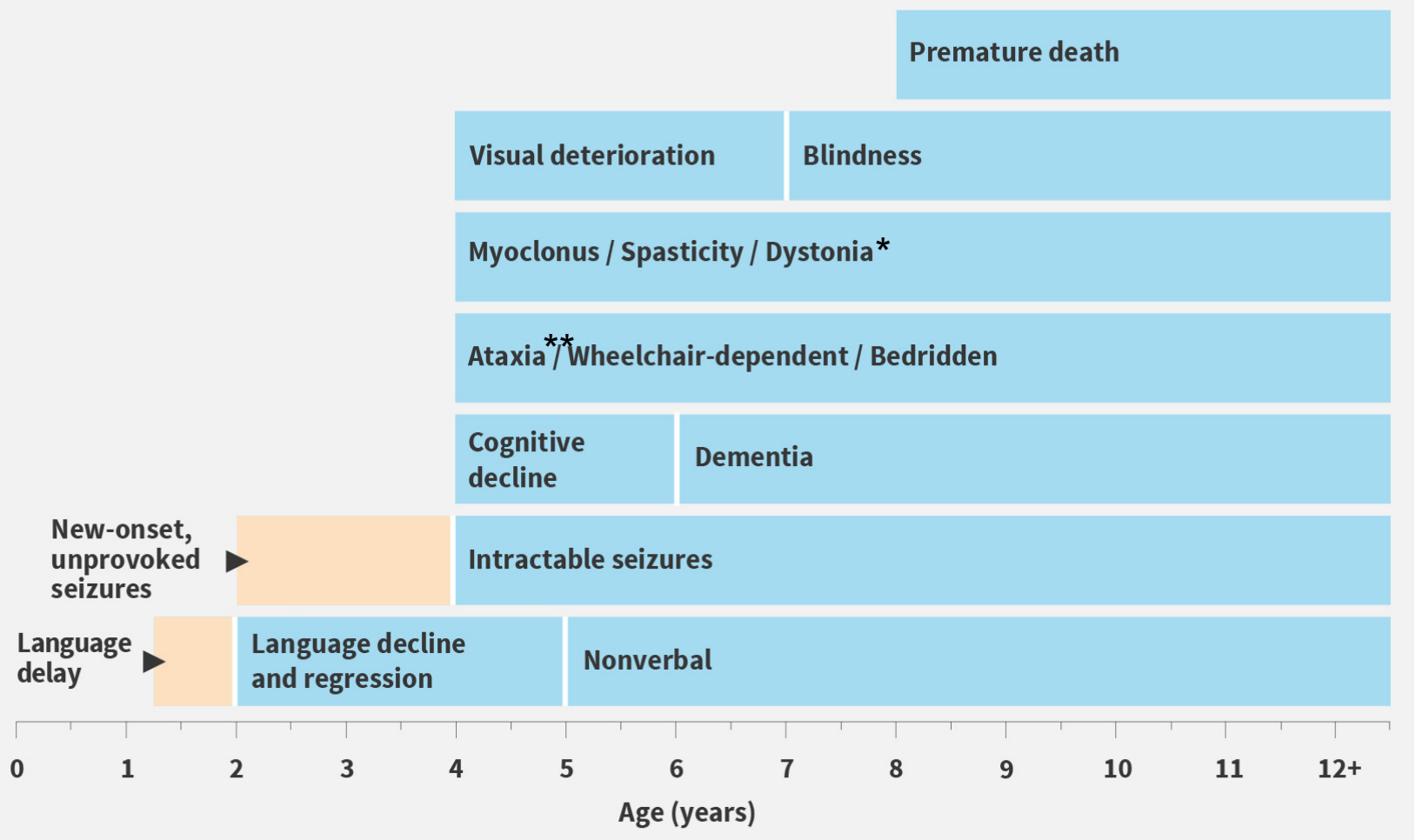
CLN2 Batten Disease Symptoms

- TPP1 expression is developmentally controlled, reaching its peak at 2–4 years of age, when the symptoms manifest[1].



Progression of Classic CLN2 Batten Disease

- Devastatingly rapid course of progression[1].
- Unprovoked seizures typically starts between ages 2 and 4
- 83% of children has delayed language development
- In some cases, other developmental delays or ataxia may be the first sign
- Symptoms and functional loss compound with age.
- Patients with small amounts of active TPP1 demonstrate slow disease progression[2].



CLN2 Disease Clinical Rating Scale

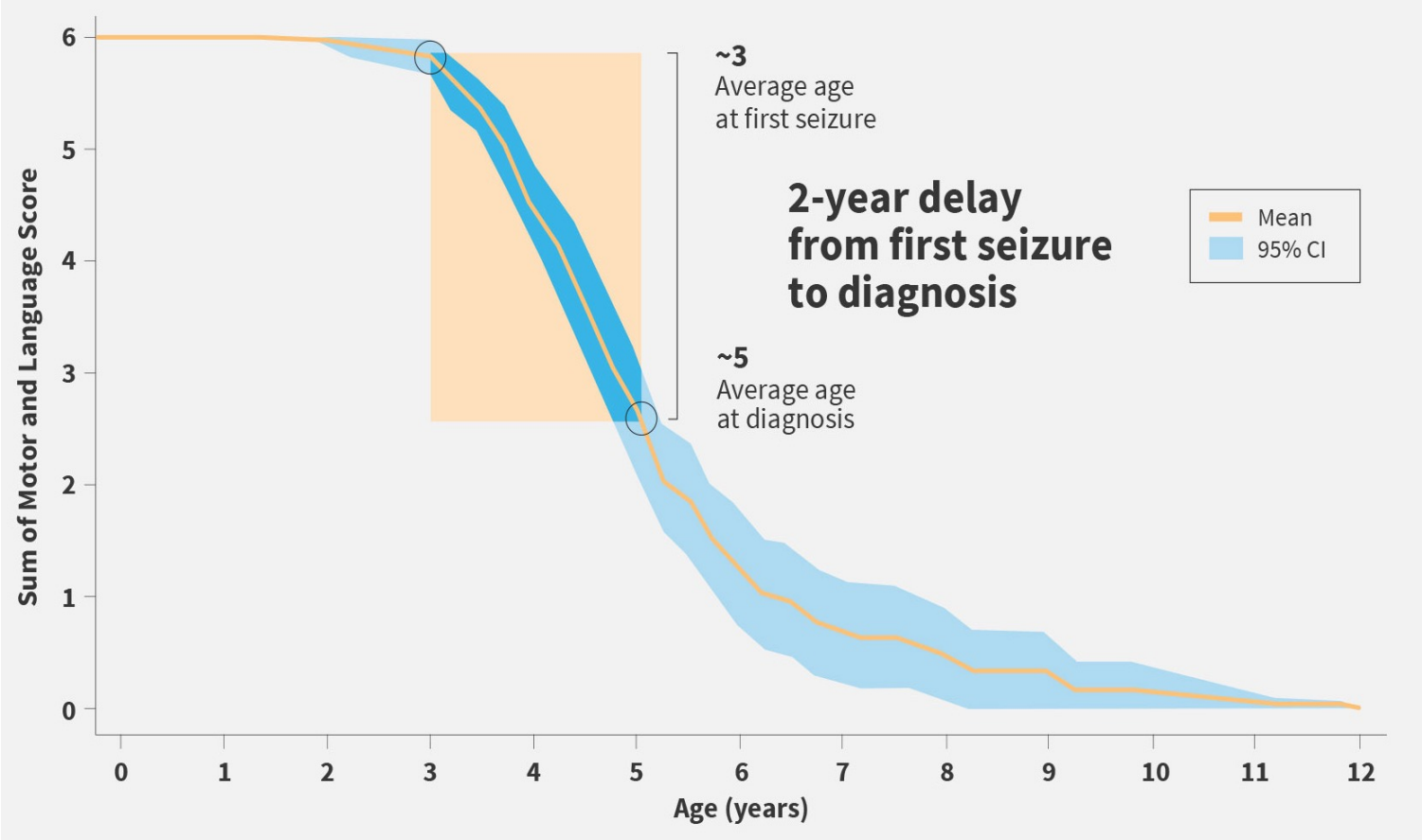
- The CLN2 Disease **Clinical Rating Scale**[1] is an efficient way to assess disease progression in 2 major functional areas: motor and language ability.
- Two additional functional domains for assessment are: vision and seizure frequency.

Motor function	Language function
3 **Normal** Grossly normal gait. No prominent ataxia, no pathologic falls.	3 **Normal** Apparently normal language. Intelligible and grossly age-appropriate. No decline noted yet.
2 **Clumsy, falls** Independent gait, as defined by ability to walk without support for 10 steps. Will have obvious instability, and may have intermittent falls.	2 **Abnormal** Language has become recognizably abnormal; some intelligible words; may form short sentences to convey concepts, requests, or needs.
1 **No unaided walking** Requires assistance to walk, or can crawl only.	1 **Minimal** Hardly understandable. Few intelligible words.
0 **Immobile** Can no longer walk or crawl.	0 **Unintelligible** No intelligible words or vocalizations.

Longer Diagnosis Compared to Disease Progression

- Consistent loss of motor and language function, as measured by the CLN2 Disease Clinical Rating Scale[1,2].

- With a rapid rate of progression, **~2 years to diagnosis** is too long:
  - Average age of first seizure: ~3 years
  - Average age of diagnosis: ~5 years
  - Average loss per year: 2 points
  - Average age of death: 10.1 years

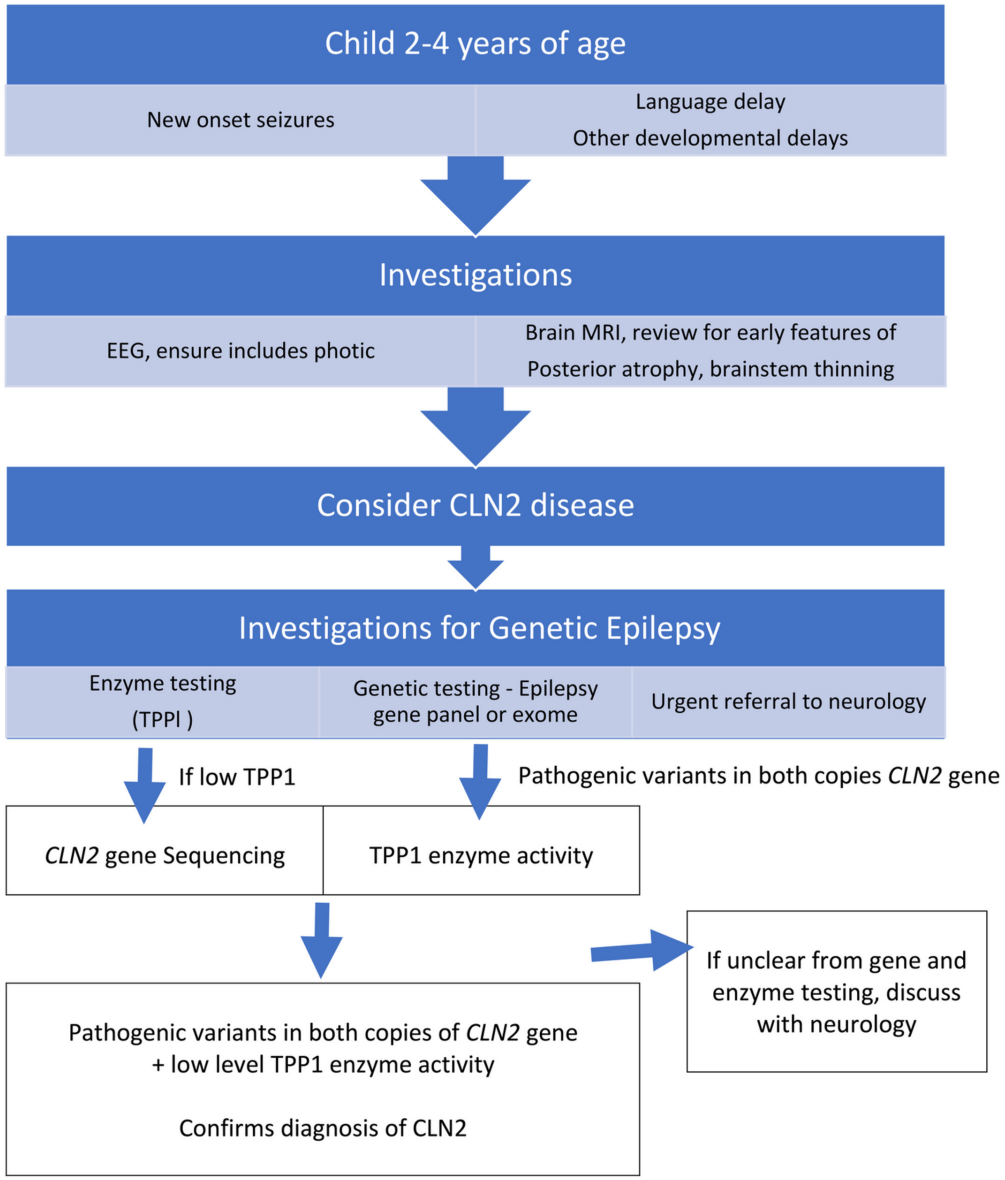


Longitudinal data from 58 subjects with CLN2 disease in DEM-CHILD registry*.
CI, confidence interval.

CLN2 Batten Disease Diagnosis

Path to Diagnose CLN2 Batten Disease

- Initial diagnosis is based on a **combination** of symptoms, ophthalmological evaluations, EEG and brain MRI[1].
- Age and onset of symptoms plays key role to direct towards confirmatory testing.
- Gold standard for confirmatory diagnosis includes molecular and/or enzymatic assessment of CLN2/TPP1.



Confirmatory Diagnosis of CLN2 Batten Disease

- **TPP1 protein assessment**:
  - Deficient TPP1 activity in leukocytes, fibroblasts, and brain homogenates.
  - Absent/reduced TPP1 using antibody-based assays.
- **CLN2 gene assessment**: The identification of pathogenic variants in both alleles of the CLN2 gene.

Two pathogenic variants are detected[1]:	• Molecular result is diagnostic
One pathogenic variant is detected[1]:	• Molecular result is not diagnostic • TPP1 enzyme test recommended
Zero, one, or two nonpathogenic variants of any kind[1]:	• Molecular result is not diagnostic • TPP1 enzyme test recommended to rule out CLN2 disease

Importance of Prenatal and Early Diagnosis

- Early diagnosis of CLN2 is critical to optimise patient outcomes which would benefit by **prenatal and newborn screening**[1].
- Children with significant speech delay or decline, clumsiness and undiagnosed/unattributed epilepsy before the age of 4 should be tested.
- **Prenatal diagnosis using electron microscopy**:
  - Examination of amniocytes reveals specific cytoplasmic inclusions, with curvilinear profiles typical of classic CLN2 disease.
  - This method may lead to misinterpretation due to the large amount of tissue debris in samples, including irregular trilaminar membranes.
- **Prenatal diagnosis using molecular analysis**:
  - Prenatal diagnosis was done by assessing TPP1 activity and CLN2 mutations, in amniocytes[2] and in first-trimester chorionic villi[3].
  - Mutation analysis with allele-specific primer extensions facilitates prenatal diagnosis, where the familial mutation is known.

Differential Diagnosis

- Batten diseases are often diagnosed too late as many neurodegenerative diseases of childhood share similar symptoms[1].
- Distinguishing epileptic seizures from nonepileptic events, including movement disorder, pain, boredom, and fear can support selection of precise medication[2].
- The differential diagnosis varies depending on disease progression.

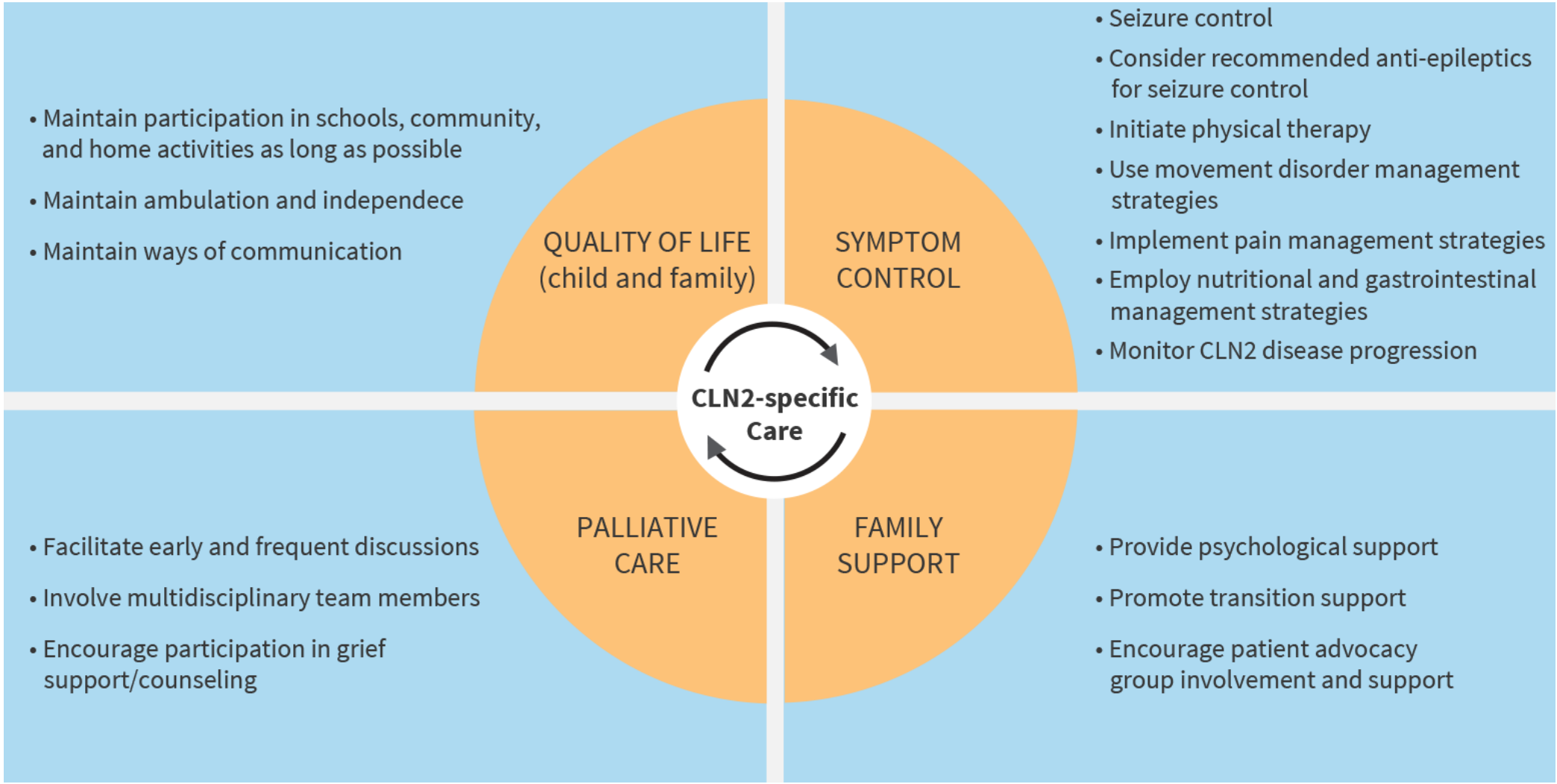
- CLN2 disease is often **NOT suspected at early stage** since seizures and myoclonus are prominent symptoms of other epilepsy syndromes and NCLs, and **at later stage** since psychomotor regression and functional loss are typical of other progressive pediatric brain disorders.

PRESENTATION	DIFFERENTIAL DIAGNOSES
A child with new-onset seizures/epilepsy [1,2]	Epilepsy syndromes (eg, myoclonic-astatic epilepsy/Doose syndrome, Dravet syndrome)
	NCLs (eg, CLN2, CLN5, CLN6, CLN7, CLN8, CLN10)
A child with progressive neurodegeneration [2]	Gray matter diseases (associated with dementia and epilepsy)
	White matter diseases (associated with motor function and significant abnormality on MRI)

CLN2 Batten Disease Management

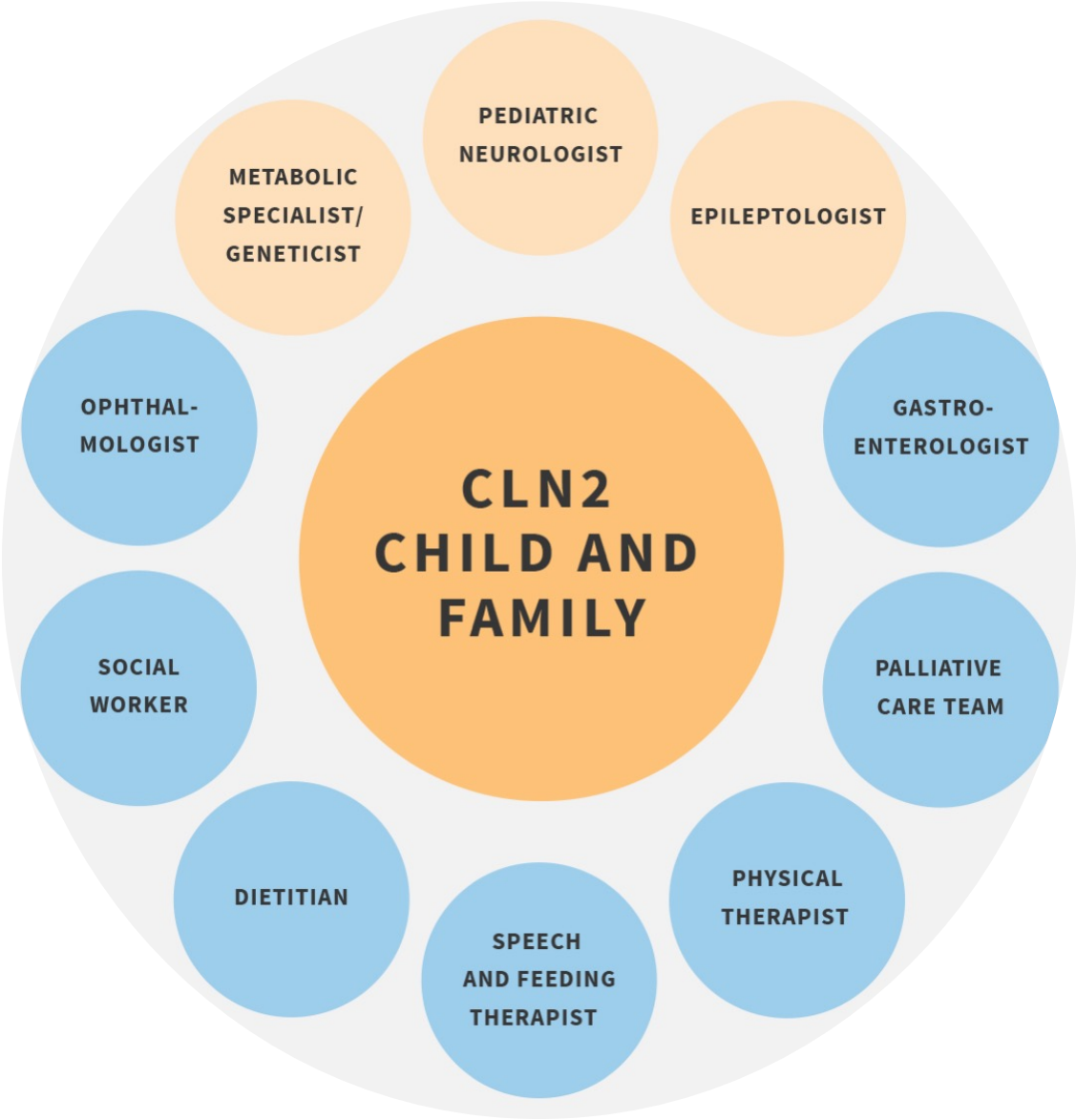
General Principles and Goals of Management

A CLN2 disease management framework for comprehensive patient- and family-centric care:



Holistic Approach for Disease Management

- CLN2 disease management support starts with a **multidisciplinary care team** to provide optimal outcomes.
- Limited access to resources in certain regions can lead to a complex diagnostic journey.
- **Reassessing the goals** of care and management is important throughout the course of the disease[1].
  - Early stage: Goals strive to maintain function for as long as possible.
  - Later stage: Goals evolve toward maintenance of quality of life and pain control as functions are lost.



Goals Evolve with Disease Progression

	Early Stage	Rapidly Progressive Stage	Late Stage
Diagnostics	• Urgency for early diagnosis • Suspect CLN2 disease in child with new-onset seizures + history of language delay/ataxia or other delays +/- PPR on 1-2 Hz IPS EEG +/- cerebellar atrophy, periventricular WM hyperintensities on brain MRI • Common misdiagnoses: non-specific language delay, non-specific epilepsy, myoclonic epilepsies	• Majority of children are diagnosed ~5 years of age (clinical suspicion is often late, not occurring until regression has occurred)	• Correct diagnosis is always important even if end-stage disease for clarity and genetic counseling
Major Management Goals	• Early diagnosis • Establish multidisciplinary care team	• Maintain function	• Maintain quality of life
Medical Management	• Symptom management • Periodic reassessment of medications • General pediatric care	• Symptom management • Ongoing assessments and modifications of treatment plans as needed • Reassessments of medications • General pediatric care	• Symptom management • Prevention of secondary complications • Ongoing assessments and modification of treatment plans as needed • Reassessments of medications • General pediatric care

Goals Evolve with Disease Progression (Continued)

	Early Stage	Rapidly Progressive Stage	Late Stage
Quality of Life	• Palliative care	• Palliative care • Maintain mobility and independence • Maintain means of communication • Maintain participation in school and community	• Palliative care • Maintain comfort • Maintain means of communication for as long as possible • Maintain participation in school and community for as long as possible
End-of-life Care	• Advance care planning with families	• Advance care planning with families	• Maximize comfort • Pain prevention and relief • Hospice care and home palliative care
Family Support	• Disease education • Family planning/Genetic counseling • Psychosocial support for parents, siblings, and other family members (grandparents) • Engagement with advocacy groups and other support services • Early palliative care team engagement	• Psychosocial support for parents, siblings, and other family members (grandparents) • Engagement with advocacy groups and other support services • Home nursing • Respite care	• Psychosocial support for parents, siblings, and other family members (grandparents) • Engagement with advocacy groups and other support services • Home nursing • Bereavement support

Nonpharmacologic Management of CLN2 Disease[1]

- No international guidelines exist for the management of CLN2 disease.
- Strategies commonly focus on a 2017 publication written by a team of CLN2 disease experts[1].

Challenges	Nonpharmacologic Management
Seizures	• Ketogenic diet • Emergency seizure management plans for home and school
Movement Disorders	• Physical and occupational therapies • Adaptive devices (gait trainers, standing device, therapy chair, lateral pillow, neck support and vests, etc.) • Ankle-foot orthoses • Frequent customization as rapid disease progression
Respiratory Problems	• Life threatening • vaccinations for children and family members against preventable respiratory diseases • regular pulmonary hygiene • Oxygen therapy

Nonpharmacologic Management of CLN2 Disease[1] (Continued)

Challenges	Nonpharmacologic Management
Communication Loss	• Speech therapy for swallowing, feeding, and communication skills • Early intervention of alternative and augmentative communication methods, such as symbols and gestures • to maintain meaningful communication after loss of speech • Complementary therapies (Hydrotherapy*, hippotherapy**) to decrease anxiety, pain, and boredom • Music therapy to comfort as hearing is preserved and other skills are lost • Patient will benefit from sensory experiences by maintaining school attendance and an Individual Education Plan
Gastrointestinal Difficulties	• Feeding and swallowing assessments • Feeding therapy • Tube feeding • Monitor nutritional status and aspiration risk*** • Manage constipation and gastroesophageal reflux

Nonpharmacologic Management of CLN2 Disease[1] (Continued)

Challenges	Nonpharmacologic Management
Sleep Dysfunction	• ~94% of patients have sleep difficulties affecting seizure, behavioral, and cognitive impairments. • Behavioral strategies (e.g., good sleep hygiene), • environmental strategies (e.g., music, massage, weighted blankets)
Visual Dysfunction	• Progressive retinal and central nervous system (CNS) pathway degeneration leads to vision loss by age 7-10 • **No interventions are currently available**
Pain and distress	• Pain assessment by Batten's Observational Pain Scale • Complementary therapies, positioning aids, weighted blankets, physiotherapy
Behavioral Symptoms	• Identifying and modifying environmental triggers • Psychology consultation
End-of-life Care	• Hospice care and home palliative care services for patients, Psychosocial support for the family

Pharmacologic Management of CLN2 Disease[1]

- Antiepileptic drugs are used for seizure management to support function (social interactions, mobility, fall prevention) while balancing the side effects (e.g., excessive sedation).
- Polytherapy is often required because of the refractory nature of the seizures.
- Patients typically take 10-12 medications daily and additional medications as needed for acute symptom exacerbations, therefore it is important to be mindful of potential drug-drug interactions.
- Medication regimes should be **re-evaluated periodically**, particularly when there is a new emerging symptom.

Symptom	Medications
Seizures	Benzodiazepines (clobazam, clonazepam), ethosuximide, lamotrigine, levetiracetam, phenobarbital, valproic acid, zonisamide; most commonly used is valproate in various add-on combinations
Myoclonus*	Benzodiazepines (clobazam, clonazepam), lamotrigine, levetiracetam, phenobarbital, valproate, zonisamide

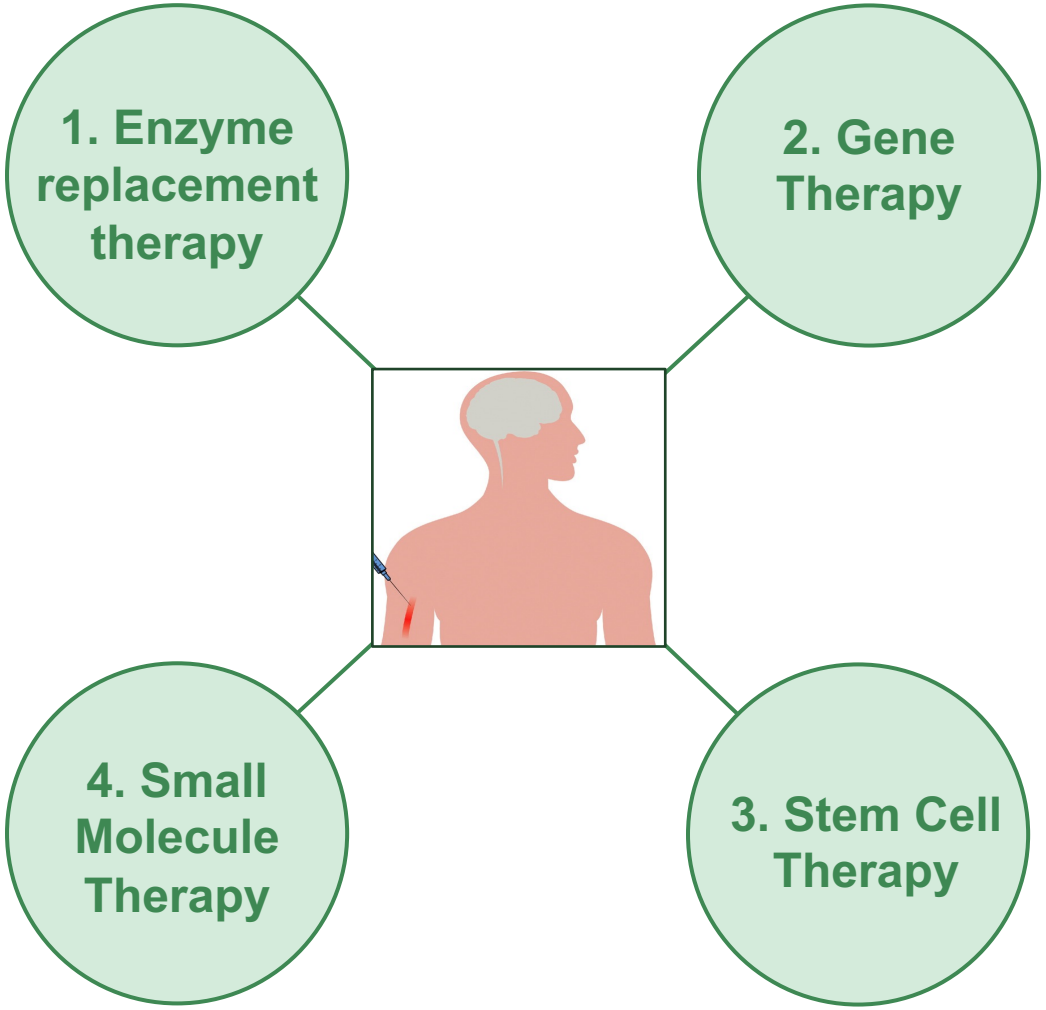
Pharmacologic Management of CLN2 Disease[1] (Continued)

Symptom	Medications
Spasticity*	Baclofen, benzodiazepines (diazepam), intramuscular botulinum toxin (focal), phenobarbital, tizanidine
Dystonia**	Baclofen, benzodiazepines, clonidine, tizanidine, trihexyphenidyl
Secretions	Enteral atropine, intraglandular botulinum toxin, glycopyrolate, inhaled ipratropium bromide, transdermal scopolamine (hyoscine)
Pain	Simple analgesia (acetaminophen, NSAIDs***); stronger analgesics (methadone, morphine, hydromorphine); others (amitriptyline, clonidine, gabapentin, pregabalin)
Breathing difficulties	Albuterol
Mucus	Dornase alfa
Sleep Dysfunction	Melatonin, chloral hydrate, clonidine, pregabalin

CLN2 Batten Disease Treatment

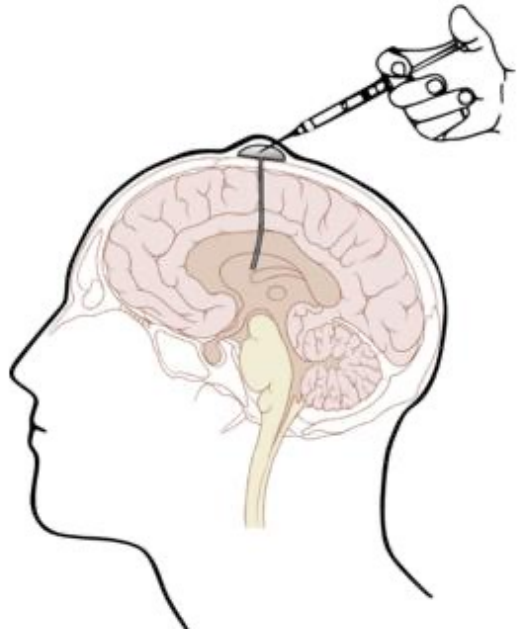
Current Treatment Landscape

- There is currently **no cure** or disease-modifying treatments that target the underlying cause of CLN2 Batten Disease[1].
- Disease is mostly managed by a combination of pharmacological and non-pharmacological based interventions[2].
- Therapeutic strategies being explored can have applications across multiple subtypes of Batten disease and lysosomal storage disorders.
- Therapeutic candidates to treat or delay disease progression by increasing brain TPP1 level belong to **4 major categories**.
- An increase in the TPP1 levels in the neurons can reduce lysosomal storage of lipofuscin leading to improvement of motor function/behavioral deficits and ultimately survival.



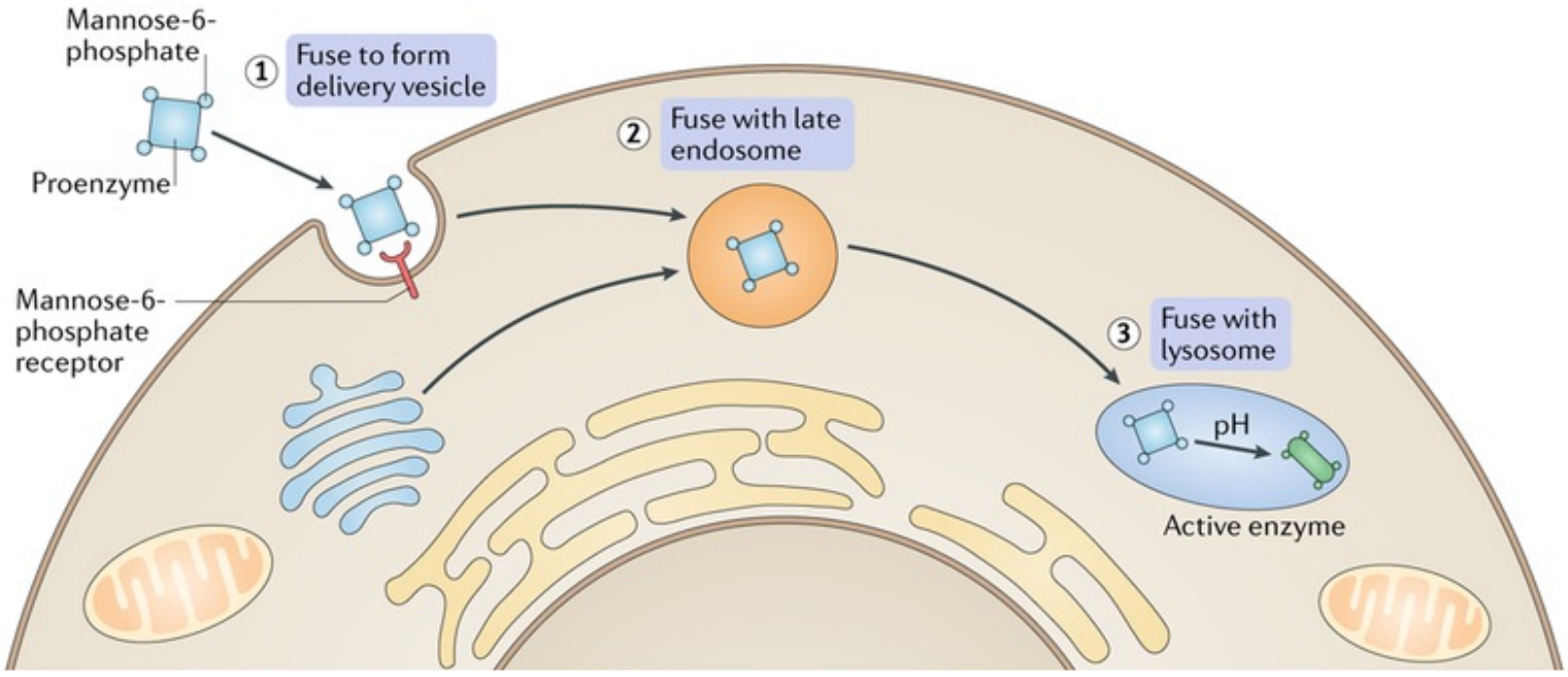
1. Enzyme Replacement Therapy (ERT)

- Enzyme Replacement Therapy (ERT) replaces an enzyme that is deficient, absent, or non-functional in the body.
- To date, cerliponase alfa, a recombinant human TPP1 protein (rhTPP1) is the only clinically approved drug for CLN2 disease.
- rhTPP1, referred to as Brineura® by BioMarin Pharmaceutical Inc. was approved in the USA for patients of ≥3 years and in Europe for all ages in 2017, and in Japan in 2019[1].
- cerliponase alfaCis delivered intraventricularly to the patient's brain.
- Doses for long-term ERT: 300 mg (or age-appropriate) dose every other week through intraventricular infusion.
- Cerliponase alfa slows the decline of motor and language function in affected children, marking the first disease-specific therapeutic advance[2].



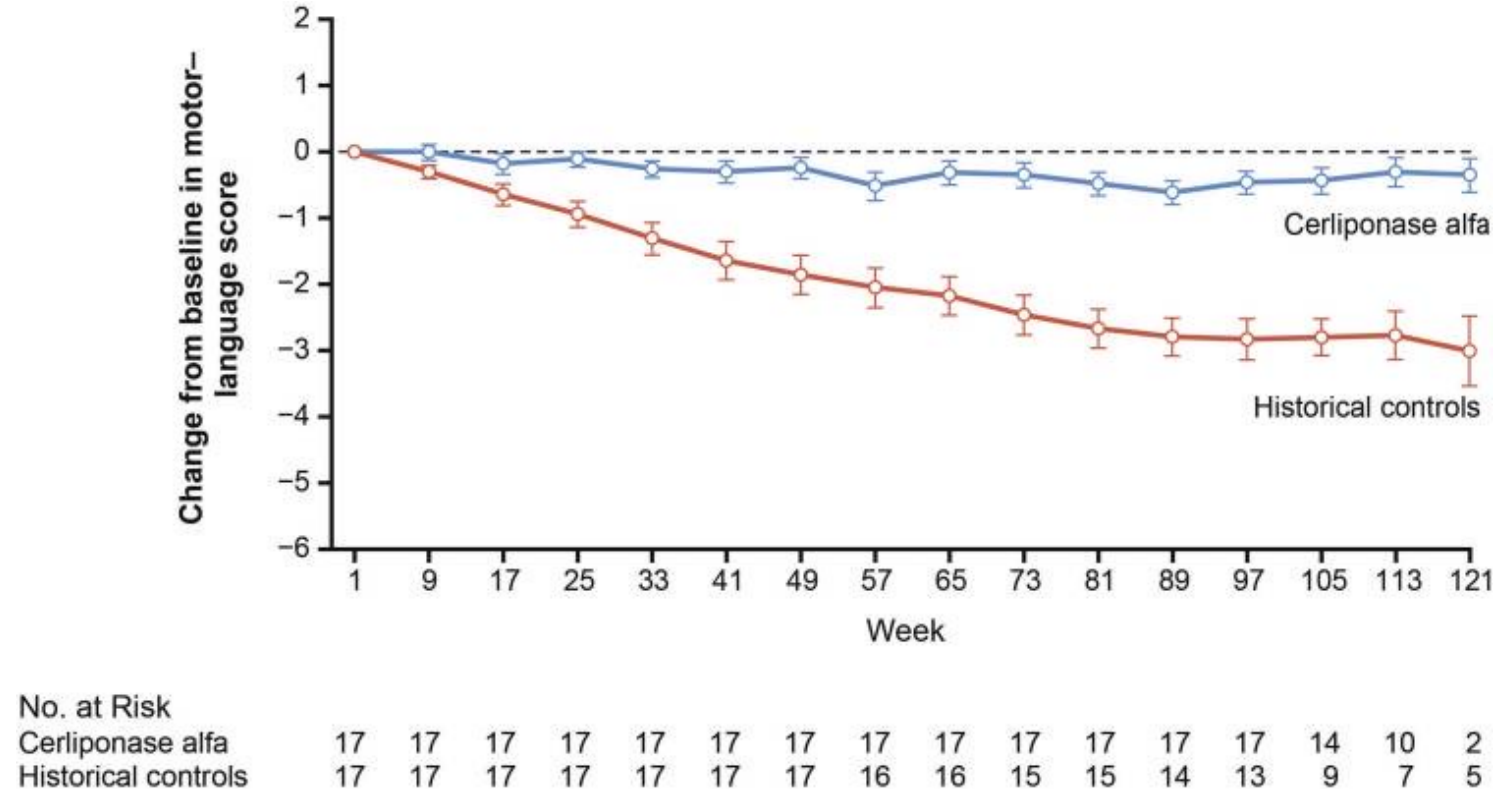
Cerliponase Alfa Mechanism[1]

- Cerliponase alfa, a recombinant proenzyme contains a mannose-6-phosphate post-translational modification.
- When delivered to brain cells, cerliponase alfa binds mannose-6-phosphate receptor on the plasma membrane and endocytosed into the cell (step 1 in figure), where it fuses with the late endosome (step 2 in figure) before being delivered to the lysosome.
- Acidic environment in lysosome converts the TPP1 enzyme to the mature, active form (step 3 in figure).



Efficacy of Cerliponase Alfa[1]

- Motor-language score declined less in treated patients compared to the historical control population: HR 0.08; 95% CI 0.02–0.23; *P* < 0.001.
- The mean motor–language score decline **1.85 points less** in treated patients than the control population (95% CI: 1.51–2.18; *P* < 0.001).



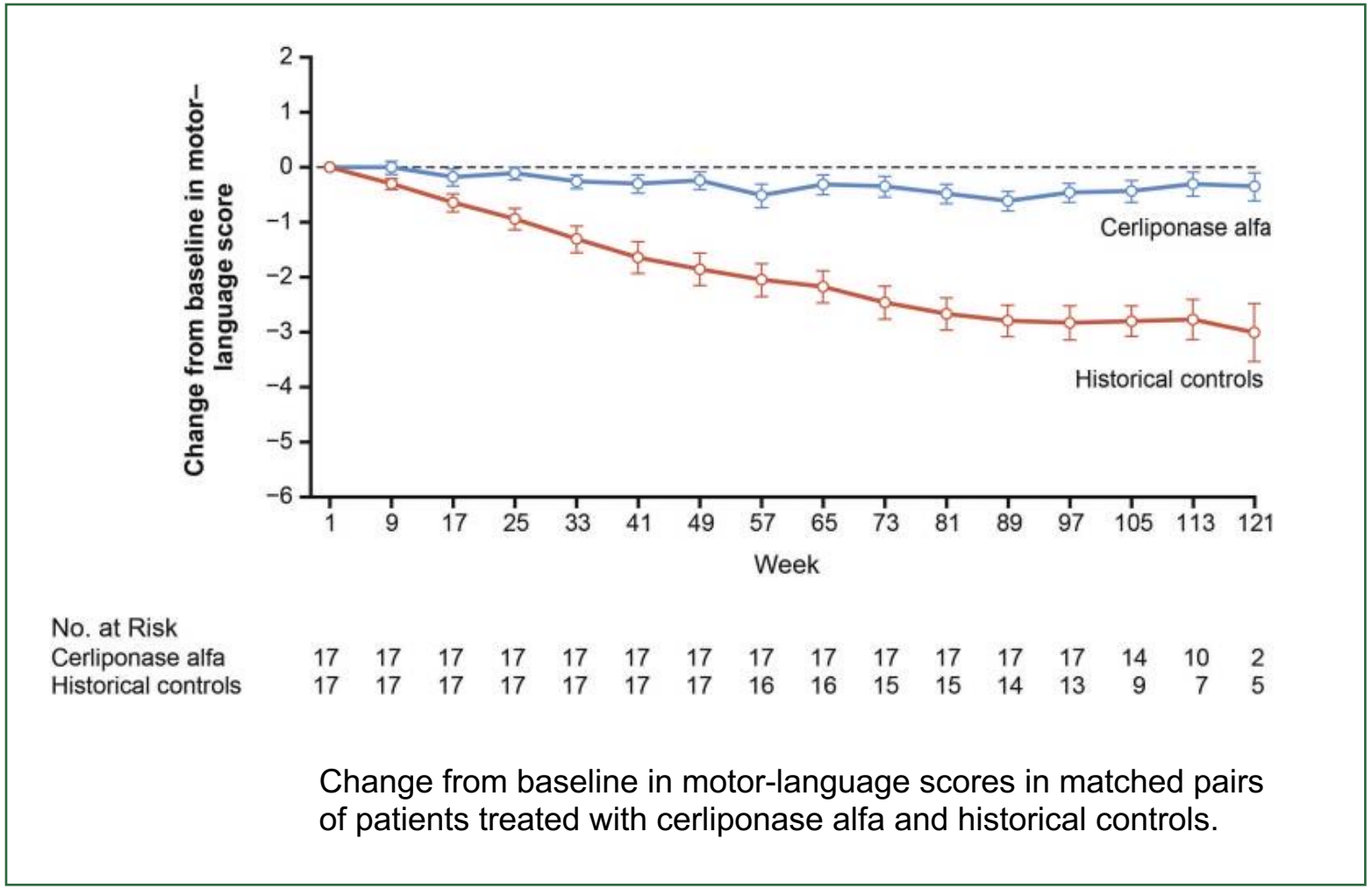
Change from baseline in motor-language scores in matched pairs of patients treated with cerliponase alfa and historical controls.

Efficacy of Cerliponase Alfa[1] (Continued)

- A small difference in clinical rating scale is very promising as it can represent the difference between walking or being wheelchair bound.
- Both motor and language scores were also affected independently:
  - Motor score:

    HR 0.04; 95% CI 0.00–0.29; *P* = 0.002.
  - Language score:

    HR 0.15; 95% CI 0.04–0.52; *P* < 0.003.



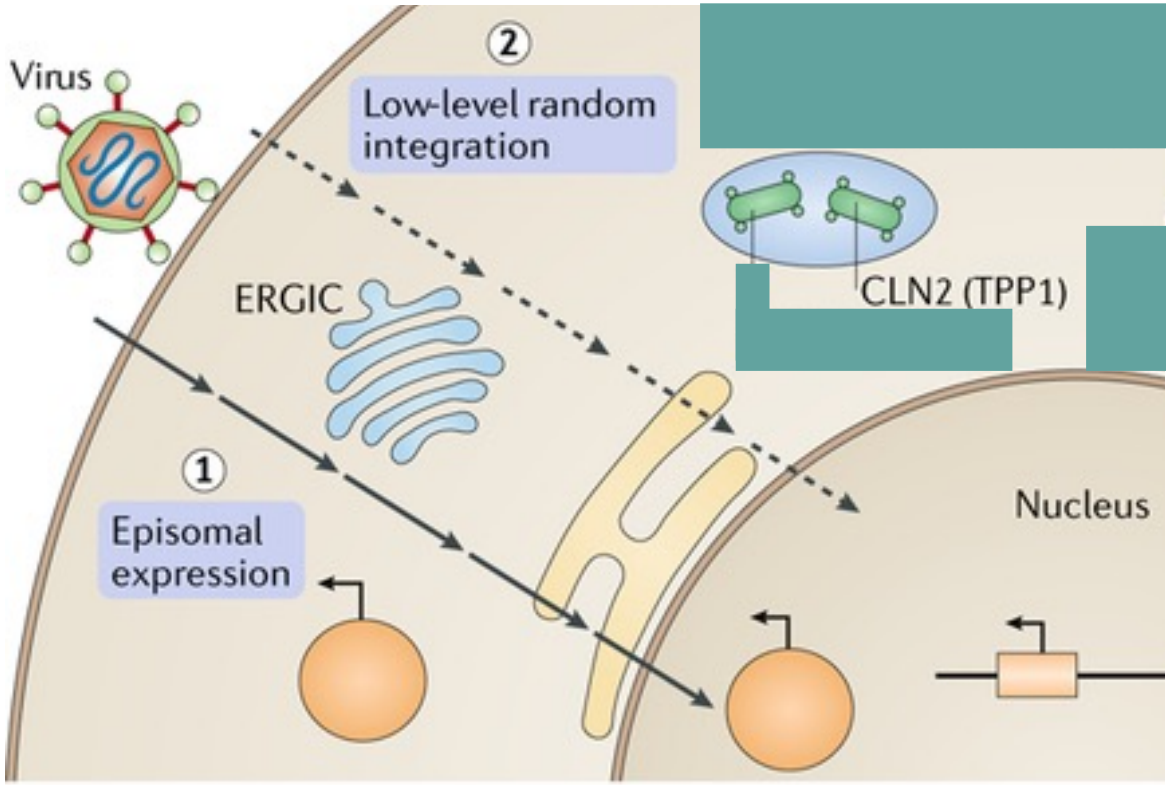
Change from baseline in motor-language scores in matched pairs of patients treated with cerliponase alfa and historical controls.

2. Gene Therapy

- Adeno-associated virus (AAV)-mediated gene therapy is a promising option for the treatment of neurodegenerative diseases and lysosomal storage disorders.
- Gene therapy has the potential to delay and prevent Batten disease pathology but not reverse it[1].
- Compared to the same control group, the gene therapy is not as effective as recombinant TPP1 therapy administered biweekly[2].

2. Gene Therapy

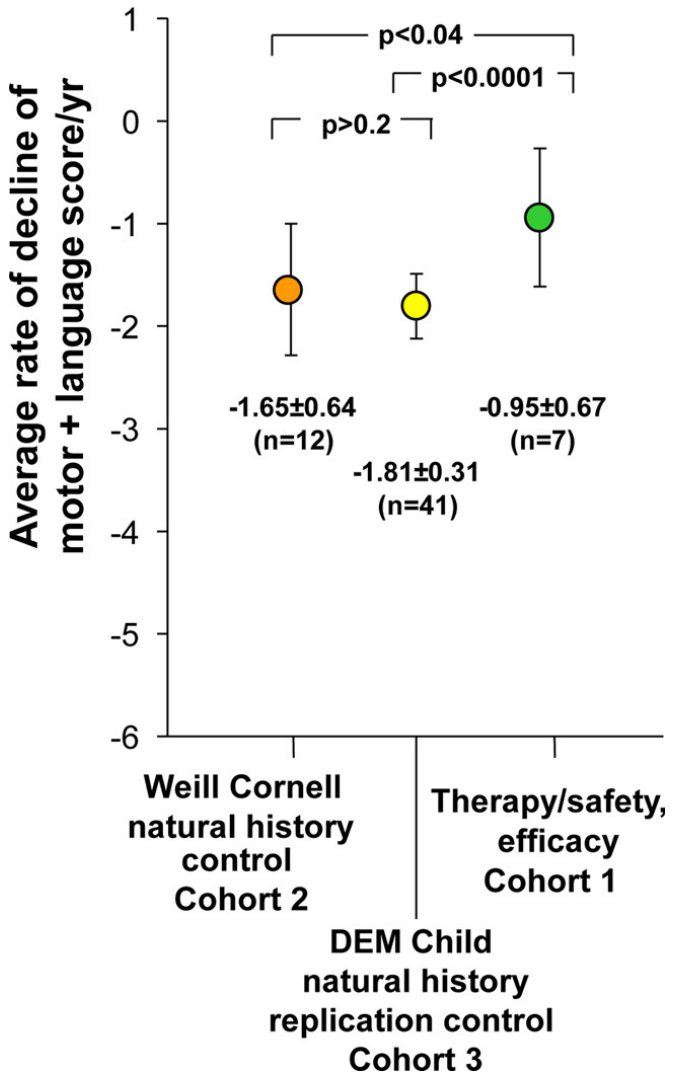
- Modified adeno-associated viruses (AAVs) target expression of the CLN2 gene to affected cells (see figure).
- Once transduced into the cell, the target transgene is episomally expressed (1) (solid arrows) or randomly integrates at the genome at low-level (2) (dashed arrows).
- Mature TPP1 translated from the transgene is trafficked to lysosome to reduce lysosomal storage of lipofuscin .



2. Gene Therapy: Clinical Trials

Clinical Trial NCT01161576

- This trial[1,2] used a similar paradigm[3], except for the use of an AAVrh.10* serotype to achieve a broader distribution of viral transduction.
- The mean motor–language score declined significantly less in the therapy cohort (cohort 1) compared to Weill Cornell natural history control cohort (cohort 2) and the DEM Child natural history control (cohort 3).
- The rates of decline/year are plotted as a mean ± SD. p values were determined using a two-tailed unpaired Student t-test.



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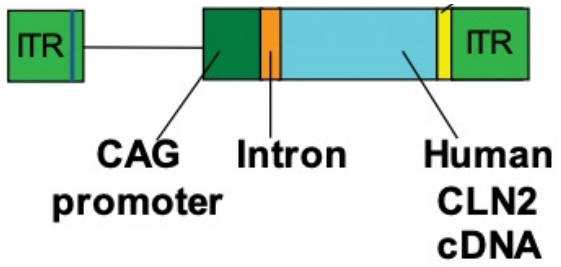
2. Gene Therapy: Clinical Trials

Clinical Trial NCT00151216

- This trial[1] assessed the efficacy of AAV serotype 2 (AAV2) expressing the human CLN2 cDNA. The vector is comprised of an AAV2 capsid encompassing a genome composed of 5' and 3' AAV2 inverted terminal repeats (ITR) surrounding CLN2 cDNA.
- Twelve intracranial injections were administered to patients of moderate or severe stages.
- Assessment at 18 months indicated that the treatment was well tolerated and the rate of decline significantly slowed when compared with the natural history studies[2]. Although gene therapy slowed down the neurological decline in the treated children, the study design lacked power.



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2. Gene Therapy: Orphan Drugs

- FDA has granted both orphan drug to RGX-181 and LX1004, two AAV-based gene therapy candidates delivering CLN2[1,2]. Orphan Drugs are drugs intended to treat a rare disease that affects <200,000 people in the USA.
- FDA has granted rare pediatric disease designations to CLN2 disease. Rare pediatric disease are life-threatening diseases that primarily affect children ≤18 years and fewer than 200,000 people in the USA.

RGX-181

- **RegenexBio** is developing a one-time gene therapy RGX-181[1].
- RGX-181 uses the AAV9 vector to deliver CLN2 gene directly to the central nervous system.

LX-1004

- **LEXEO Therapeutics** is developing LX1004, an AAV-based gene therapy candidate delivering CLN2[2].
- Phase I clinical trial is complete.
- Phase II clinical trial is planned.

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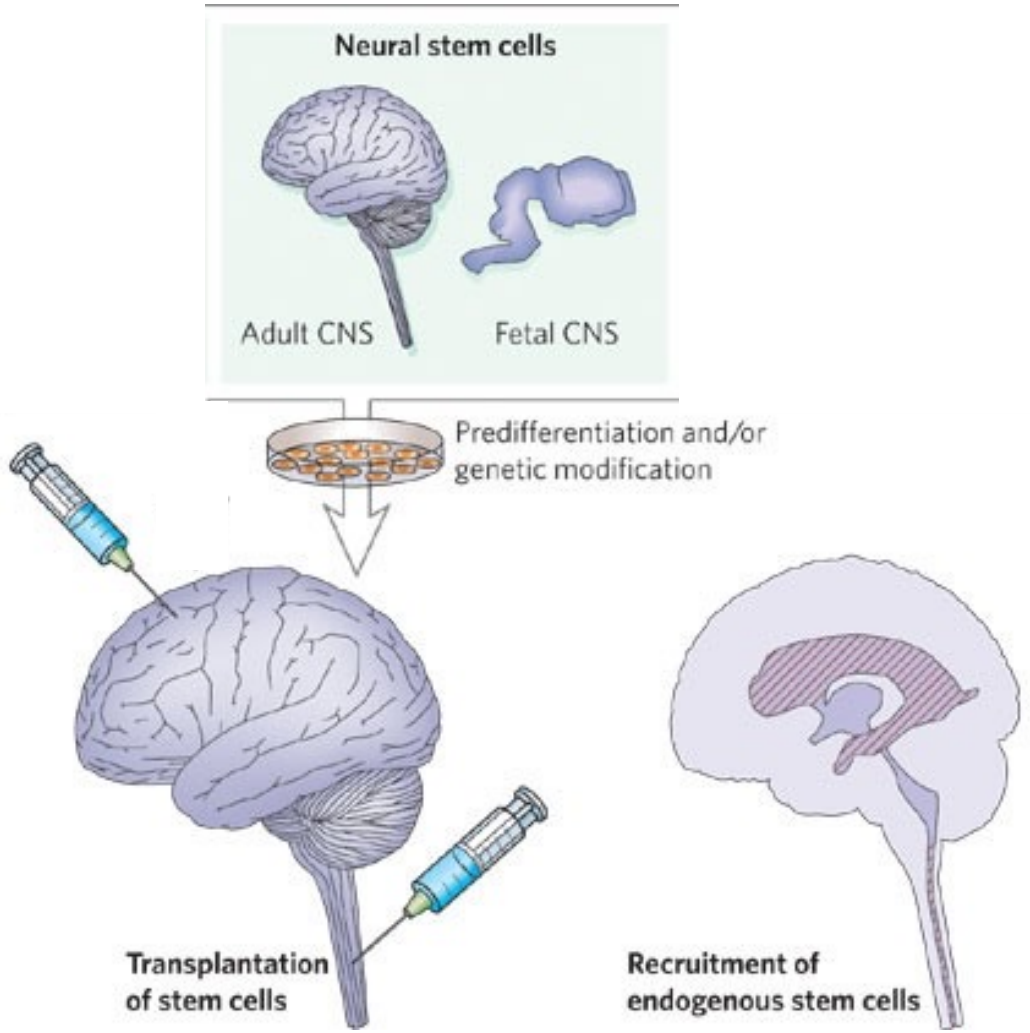
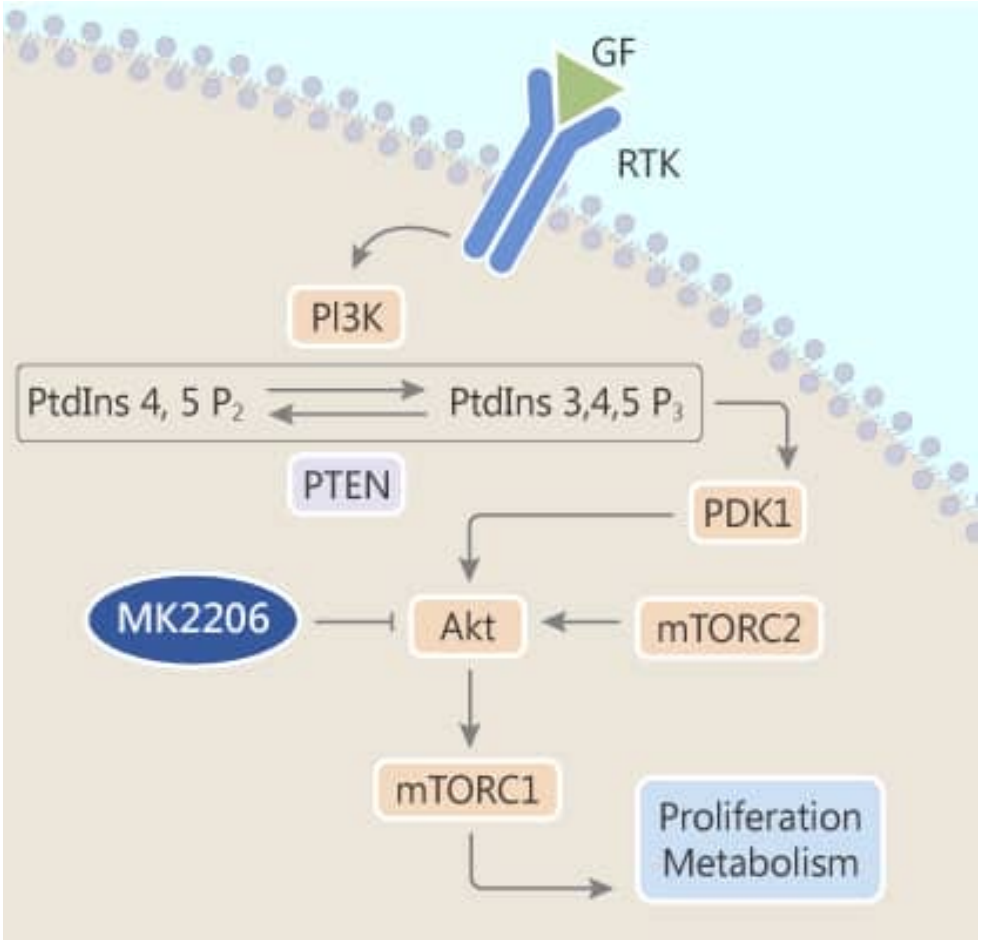
3. Stem Cell Therapy

- Stem cell transplantation from a healthy donor into patients' brain provides a source of functional TPP1.
- Stem cell therapy offers the potential to regenerate lost tissue alongside neuroprotection.
- In a clinical trial[1], human CNS-derived stem cells that secreted endogenous TPP1 were engrafted into the cerebral hemispheres and lateral ventricles of 4 advanced-stage CLN2 patients.
- The treatment was safe and well tolerated but did NOT slow disease progression in treated patients compared with the natural history controls
- However, post-mortem PCR analysis of samples from two of the patients detected engrafted stem cells 357 days and 918 days after stem cell injection, indicating integration and longevity of stem cells after engraftment.



4. Small Molecule Therapy

- Based on Batten disease pathogenesis, preclinical approaches have focused on small molecules that improve lysosomal or autophagic health, serve as immune modulators or neuroprotective agents, or increase transcript or protein abundance.
- **Gentamicin**, an aminoglycoside antibiotic that binds to the 30S ribosomal subunit, successfully increased TPP1 transcript levels and enzyme activity in cell lines from CLN2 patients[1]. However, this has not been tested in mouse models of Batten disease to date.
- **MK2206** is an autophagy inhibitor[2]. It is a selective allosteric Akt inhibitor and has shown to reduce storage material in fibroblasts from patients with CLN2 and other NCLs[1].



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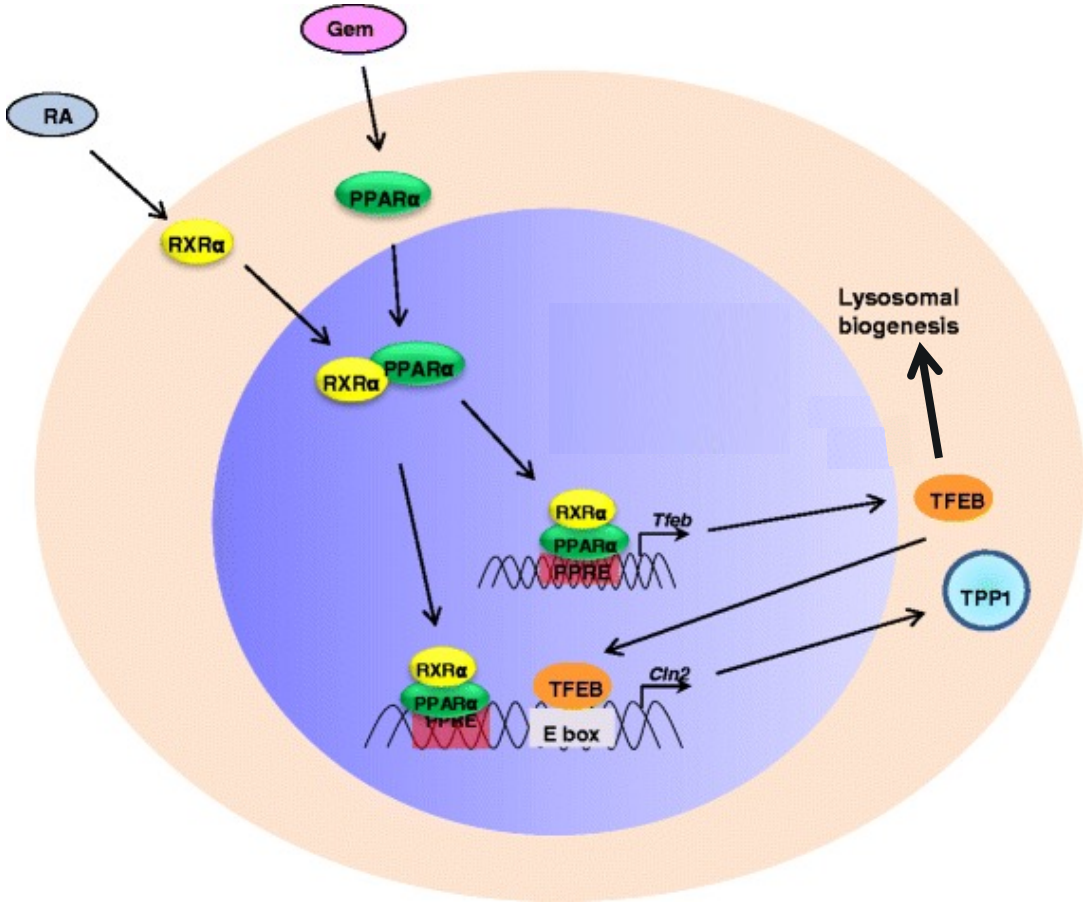
4. Small Molecule Therapy

Other Autophagy Modulators

- Immature autophagosome formation and improper autophagosome–lysosome fusion have been found in mouse models of Batten diseases and other neurodegenerative diseases.
- PPARα enhances TFEB expression, which then binds to promoters of lysosome biogenesis genes to increase their expression.
- The combination of gemfibrozil[1] (a PPARα agonist) and retinoic acid forms PPARα/RXRα heterodimer and promotes TFEB expression in cultured fibroblasts from CLN2 patients.
- FDA designated this combination drug (**PLX-100 by Polaryx Therapeutics**) as an orphan drug in 2017.



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Thank you!

Tania Sultana, PhD

Tania.sultana@mednarrate.ca