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# Gene therapy for Batten disease: A systemic review of preclinical and clinical studies

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### *Abstract*

*Background:* Batten disease (BD), or neuronal ceroid lipofuscinosis (NCL), is a group of rare, fatal neurodegenerative disorders caused by mutations in CLN genes, leading to lysosomal dysfunction and progressive neuronal loss. Gene therapy, particularly using adeno-associated virus (AAV) vectors, has emerged as a promising strategy to address the genetic basis of BD across various subtypes, including CLN2, CLN5, and CLN6. This systematic review evaluates the efficacy and challenges of gene therapy in preclinical and clinical settings. *Methods:* Following PRISMA guidelines, we reviewed studies from January 2000 to January 2025, sourced from PubMed, Embase, Scopus, and other databases. Included studies assessed viral and non-viral vector-mediated gene therapies for BD in preclinical (animal, in vitro) and clinical contexts. Primary outcomes were restoration of enzymatic function, reduction in neurodegeneration, improvements in motor and cognitive function, safety, and survival. *Results:* Preclinical studies demonstrated that AAV-based gene therapy effectively restored enzyme activity, reduced neuronal degeneration, and extended survival in models of CLN5, CLN6, and other subtypes. Clinical trials, particularly for CLN2, showed slower disease progression with intracerebroventricular AAV-TPP1 delivery. However, challenges include genetic heterogeneity, immune responses to vectors, limited central nervous system (CNS) transduction due to the blood-brain barrier, and uncertainties about long-term safety and optimal treatment timing.
*Conclusion:* Gene therapy holds significant potential for treating BD by targeting its genetic roots, with AAV-mediated approaches showing promise in both preclinical and early clinical studies. Nonetheless, optimizing vector design, delivery methods, and immune management, alongside improving early diagnosis, remains critical to realising its therapeutic potential.

*Keywords:* Batten disease, neuronal ceroid lipofuscinosis, gene therapy, lysosomal storage disorder, CLN genes, AAV vectors, preclinical studies, clinical trials.

## INTRODUCTION

Batten disease (BD), also called neuronal ceroid lipofuscinosis (NCL), is a very rare and always fatal neurodegenerative disease that mainly attacks children. Characterised by progressive deterioration of the nervous system in the form of visual loss, cognitive impairment, motor defects, and seizures, the disease results in mutations in one of at least 14 genes called ceroid lipofuscinosis neuronal (CLN) genes. The mutations disrupt functions of the lysosomes and result in an increase in lipofuscin, which is a toxic compound in the cells of the neurons.[1-4]

The clinical presentation of BD is highly variable depending on the type of genetic mutation. Loss of vision is usually the first symptom that presents, followed by a decline in cognitive abilities and motor inefficiencies. There may be seizures later, and ultimately, the patient will become unable to communicate or perform activities of daily living, becoming bedridden and premature death.[5-7] The estimated incidence of BD is between 2 to 4 cases per 100,000 live births, therefore being one of the most common neurodegenerative diseases in childhood.[8,9]

Therefore, BD pathophysiology is a complex play of genetic and cellular mechanisms. Mutations within the CLN genes lead to dysregulation of lysosomal function; this is an important process in which cells handle their waste. Dysfunction will lead to the accumulation of undigested material in the lysosomes and, therefore, neuronal degeneration.[7,10,11] Other features include neuroinflammation and changed

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secretion of proteins, which will further accelerate the damage to the neurons.[7,12]

The present study focused on developing opportunities for the treatment of BD in the form of enzyme replacement therapy, gene therapy, and pharmacological intervention. These are to reverse genetic defects that cause disturbances in lysosomal function.[13,14] However, more will be required to be done in terms of effective and advanced treatment that will be able to halt the disease process and usher in a good quality of life in the patients.[15]

One of the best treatments for CLN2 is enzyme replacement therapy. This disease is caused by the deficiency of an enzyme called tripeptidyl peptidase 1 (TPP1). Cerliponase alfa is a recombinant form of TPP1 and has been cleared by the FDA for use in CLN2. This treatment is intracerebroventricular and has been demonstrated to effectively stop the disease's progression, particularly in the preservation of motor and language functions in the children afflicted by Sondhi *et al*.[16,17] The clinical studies have also established that the patients treated with this drug showed a drastic reduction in neurological deterioration as compared to the patients who did not receive the treatment.[5,18]

Other than enzyme replacement, gene therapy is also finding its role in the treatment of nearly all subtypes of BD. For instance, promising early data from using adeno-associated virus vectors have been established for several of the subtypes, including CLN3 and CLN6, in preclinical models.[15,19] Gene therapy introduces functional copies of the mutated genes directly to the CNS, directly correcting the genetic cause of the disease. This was the most commonly noted AAV9, known for its capability to deliver therapeutic genes across the blood-brain barrier.[19]

Small molecule therapeutic candidates have also been explored in research studies. For example, a small molecule enzyme mimetic N-Tert-Butyl

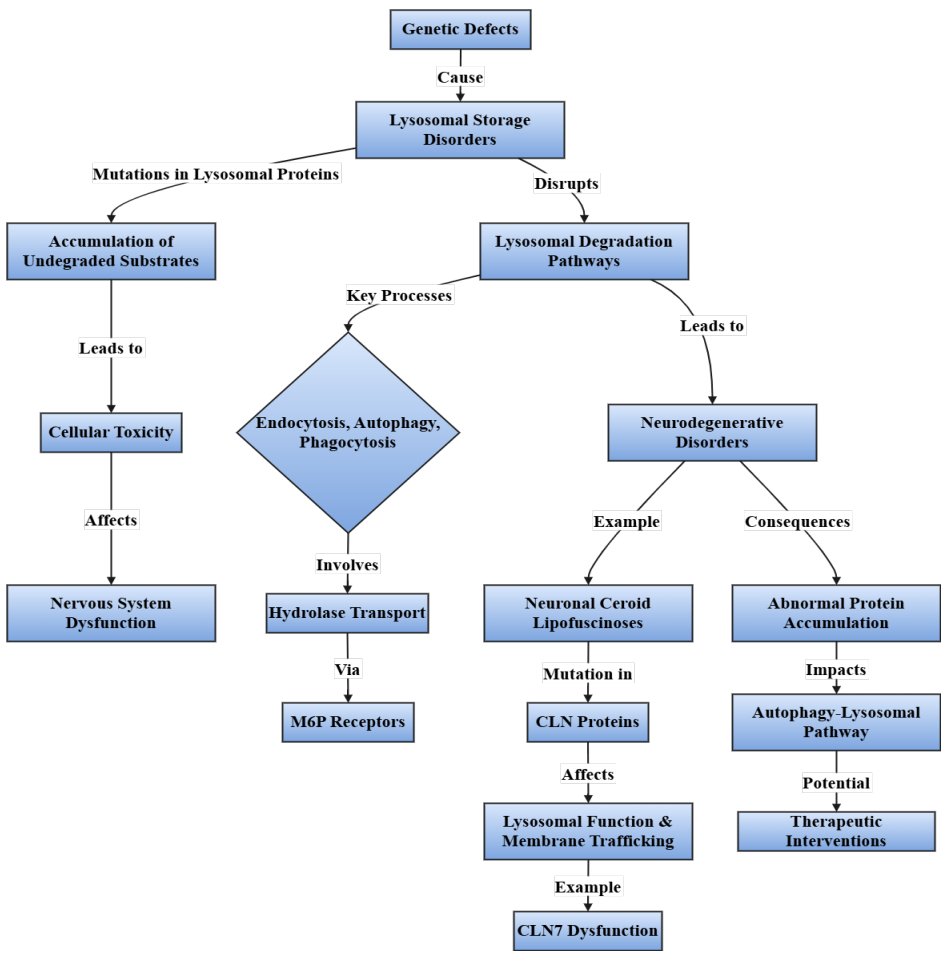


Figure 1: Lysosomal pathways and CLN proteins

Hydroxylamine reduced neuroinflammation and alleviated seizures in animal models of CLN1 BD.[12] This may lead to certain alternative treatments using pharmacological therapies targeting the specific pathways involved in the disease pathology.

Supportive care is the backbone of the management of BD. These include antiseizure medications to manage seizures, physical therapy to preserve mobility, and psychological support for families and patients.[5,6] All these treatments contribute to the enhancement of the quality of life of the patient, even in the absence of curative therapies.

The biggest challenge lies in the heterogeneity of BD, which has several subtypes due to mutations in different genes (CLN1-14). Each subtype has varied clinical features and varying rates of progression, so different therapeutic approaches must be used for each subtype.[3,20] Such genetic heterogeneity increases the difficulty in devising a single gene therapy that will fit all because each mutation may require specific vectors and delivery methods to restore normal function.[3,16] For instance, whereas some of the subtypes of BD may respond favourably to AAV vectors, others may not due to the variation in uptake and expression between cells.[21,22]

Another significant hurdle is the efficient delivery of gene therapy to the CNS. The blood-brain barrier (BBB) has limited systemic delivery of therapeutic agents due to its function as a difficult barrier to permeate into the brain and spinal cord.[14,19] Although AAV vectors have been promising in preclinical models for CNS disorders, the distribution and effective transduction of target cells remain challenging.[21,23] Moreover, the route of administration (e.g., intrathecal, intracerebral) may also influence the effectiveness of gene therapy; targeted delivery has improved outcomes in animal models in some studies.[24,25]

In addition, the time of intervention is critical. BD is a progressive illness, and early intervention may be crucial to prevent irreversible neuronal cells damage.[10] However, there is variability in the onset and progression of the symptoms among patients, thus making it challenging to define a precise window period for initiating treatment.[10,20] There is a potential for missed opportunities for effective treatment with delayed diagnosis; this has raised concern over improving public awareness and screening for early signs of disease.[26]

Another difficulty for gene therapy is the generation of immune responses against the viral vectors used. Patients will develop antibodies against AAVs, which would serve to neutralize the therapeutic effect of subsequent administrations.[21,22] Such an immune response can limit the effectiveness of the therapy and complicate the design of multi-dose regimens necessary for sustained treatment.

Finally, the long-term consequences and safety of gene therapy concerning BD are yet to be determined. Even if primary research is promising

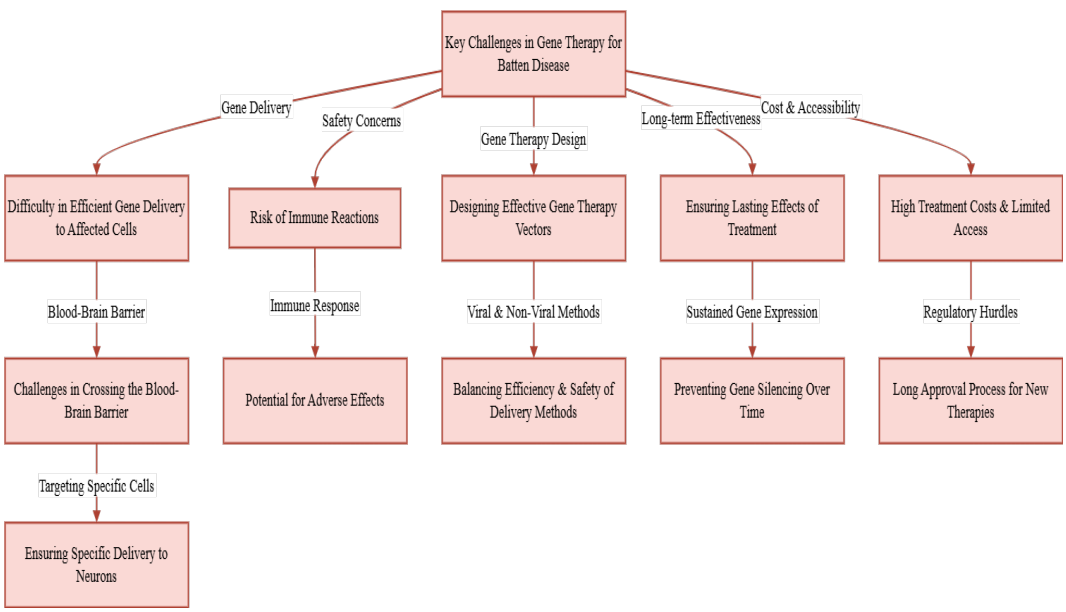


Figure 2. Key challenges in developing gene therapy for BD

at first, significant reviews regarding the possibility of insertional mutagenesis and off-target effects must be conducted to safeguard the patient.[16,21]

## METHODS

*Review design*

*This systematic review's PECOS protocol*
(Population, Exposure, Comparison, Outcomes, Study design) followed the reporting guidelines of PRISMA so that it can be made transparent and reproducible.

*Database search protocol*

To ensure an all-inclusive capture of literature, a database search strategy was conceptualized. The databases searched include PubMed, Embase, Scopus, Web of Science, Cochrane Library, CINAHL, and PsycINFO. The precision of the search was maximized using Boolean operators and MeSH keywords. It included combinations such as:

"Gene Therapy" OR "Gene Transfer Techniques" OR "Gene Editing "OR "Gene Delivery" OR "Gene Therapy") AND ("BD" OR "Neuronal Ceroid Lipofuscinosis" OR "NCL" OR "CLN1" OR "CLN2" OR "CLN3" OR "CLN5" OR "CLN6" OR "CLN7" OR "CLN8" OR "CLN10")) AND ("Preclinical Study" OR "Animal Model" OR "Clinical Trial" OR "Phase I" OR "Phase II" OR "Phase III")

*Data extraction protocol and data items*

Application of data extraction was conducted by using a form of pre-designed data extraction. Data extraction used two independent reviewers to limit error and bias. Included are study characteristics such as author, year, location, study design, samples of size, and demographics; Gene therapy details such as vector type, delivery method, dosage, Outcomes; efficacy, adverse events, survival, quality of life major findings; the statistical outputs that included odd ratios or beta coefficients; environmental exposures examined. Third, reviewers compared and resolved inconsistencies by cross-checking in consensus.

## RESULTS

The systematic review included a diverse set of preclinical and clinical studies evaluating gene therapy approaches for BD. The included studies covered various CLN subtypes, with a predominant focus on AAV-mediated gene therapy.

**Table 1: Inclusion and exclusion criteria devised for this review**

| Criteria | Inclusion | Exclusion |
|---|---|---|
| **Population** | Preclinical (animal, in vitro) and clinical studies on BD (all genetic subtypes), including paediatric/adult patients receiving gene therapy. | Studies on other neurodegenerative diseases or mixed populations without separate BD analysis. |
| **Exposure** | Viral and non-viral vector-based gene therapy, gene replacement, silencing, or modification. | Non-gene therapy approaches, gene therapy for other diseases, and combination therapies. |
| **Comparator** | Untreated/placebo groups, other interventions, healthy controls, historical controls (if RCTs are unavailable). | No control/comparator group or irrelevant comparators. |
| **Outcomes** | Preclinical: Gene expression, enzyme activity, neurodegeneration reduction, motor/cognitive improvements. Clinical: Neurological function, disease progression, survival, safety, biomarkers. | Studies lacking efficacy, mechanistic/ genetic findings only. |
| **Study design** | Preclinical and clinical studies. | Reviews, commentaries, computational/ in silico studies without validation, non-Batten models. |
| **Publication language** | English only. | Non-English publications. |
| **Publication year** | Studies published from Jan 2000 to Jan 2025. | Studies published more than 25 years ago. |

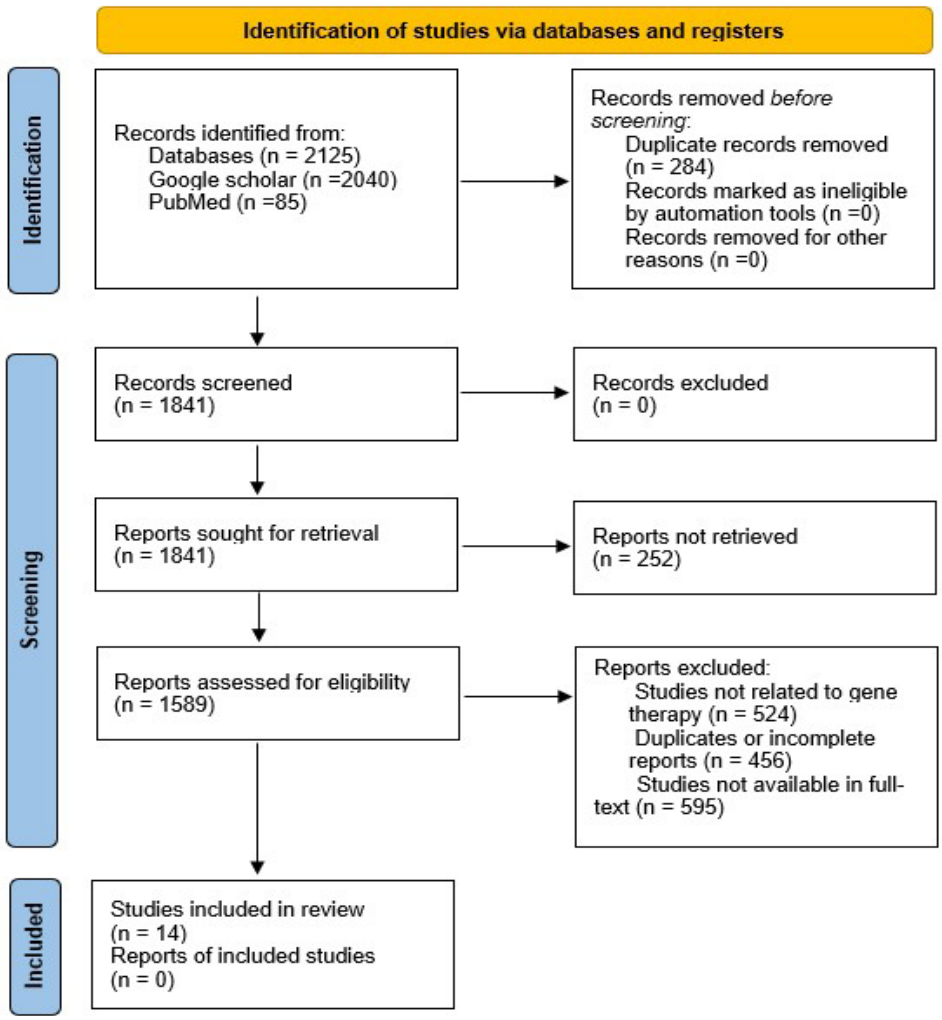


Figure 3: PRISMA flowchart

*Preclinical findings*

Preclinical studies supported that AAV-based gene therapy successfully restored enzymatic function, reduced neuronal degeneration, and improved survival rates in animal models. Studies using CLN6 and CLN5 gene therapy in mice and sheep reported significant neurological improvements and extended lifespan. Importantly, the systemic and intracerebroventricular routes of delivery show promising efficacy for widespread CNS distribution. In addition, gene therapy of retinal degeneration in models of BD has provided neuroprotection with preservation of visual function.

*Clinical finding*

Early Phase 1 trials of safety and efficacy have been obtained. The disease has progressed with slower advancement by gene therapy to CLN2 disease after the intracerebroventricular administration of the recombinant TPP1. For CLN5 and CLN6, clinical results for long-term therapy are also suggestive, and future larger cohorts, along with more extensive follow-up, should be followed in order to obtain the confirmatory therapeutic benefits.

Despite these encouraging results, major issues arise: heterogeneity of the response to therapy, immune reaction to the vectors, and adequate dosing strategy. These are some of the reasons that give more value to continued research and optimization in gene therapy to be used for treating BD.

*Assessment of bias*

The risk of bias assessment, conducted using the

**Table 2: An outline of gene therapy strategies for BD (CLN Disorders)**

| Study | Disease Type (CLN) | Gene Target | Delivery Method | Sample | Major Findings | Adverse Effects |
|---|---|---|---|---|---|---|
| **Holthaus *et al*., 2019[27]** | CLN6 | CLN6 gene | Neonatal brain-directed | Mice | Rescued a mouse model of neurodegenerative CLN6 BD | Not specified |
| **Mitchell *et al*., 2023[28]** | CLN5 | CLN5 gene | Intracere broventricular | Sheep | Long-term safety and dose escalation support clinical translation | Well-tolerated |
| **Cain *et al*., 2019[29]** | CLN6 | CLN6 gene | Systemic | Mice | Corrected brain and behavioural pathologies | None reported |
| **Brudvig & Weimer, 2022[15]** | CLN7 | CLN7 gene | Not specified | Not specified | Offers hope for treating this ultra-rare condition | Not specified |
| **Mitchell *et al*., 2018[30]** | CLN5 | CLN5 gene | In Vivo Monitoring | Sheep | Demonstrates efficacy of gene therapy | Not specified |
| **White *et al*., 2021[23]** | CLN6 | CLN6 gene | Intracranial AAV9 | Mice | Partially prevents retinal degeneration and visual deficits | Minimal |
| **kleine Holthaus *et al*., 2020[31]** | CLN3 | Inner retina | Gene therapy targeting | Mice | Rescues retinal phenotype | Not specified |
| **Sondhi *et al*., 2020[16]** | Late infantile | CLN2 | Direct brain parenchymal (rh.10 AAV) | Not specified | Slows the progression of the disease | Not specified |
| **Johnson *et al*., 2020[32]** | CLN8 | AAV9 | Systemic | Mice | Restores lifespan and treats pathological and behavioural abnormalities | Not specified |
| **Murray *et al*., 2023[33]** | CLN5 | CLN5 gene | Dual intracere broventricular | Sheep | Efficacy prompts first clinical trial | Not specified |
| **Murray *et al*., 2021[34]** | CLN5 | Intravitreal | Gene therapy | Sheep | Protects against retinal dysfunction and degeneration | Not specified |
| **Katz *et al.,* 2015[35]** | Late infantile | TPP1 | AAV gene transfer | Canine | Delays disease onset in a canine model | Not specified |
| **kleine Holthaus *et al*., 2018[36]** | General Batten | Not specified | Gene therapy approaches | Not specified | Discusses approaches to treat neurodegeneration and visual failure | Not specified |
| **Johnson *et al*., 2023[37]** | CLN3 | AAV9 | Early postnatal administration | Not specified | Safe and efficacious in treating CLN3 disease | Safe |

ROBINS-I tool, evaluated seven key domains across multiple studies. Most studies exhibited a moderate risk of bias due to confounding, with Sondhi *et al*.[16] showing a serious risk in this domain. Patient selection and intervention classification were steadily rated as low risk across all studies, showing robust methodology in these aspects. Bias due to deviations from intended interventions was also uniformly low. However, bias due to missing data was moderate in studies such as Murray *et al*.[34], White *et al*.[23], and Johnson *et al*.[32,37], suggesting potential concerns regarding incomplete outcome data. Measurement bias was a significant concern in Sondhi *et al*.[16] and White *et al*.[23], where a serious risk was noted, possibly due to issues with outcome assessment or lack of blinding. Despite these issues, bias in choosing reported results was reliably low for all studies. Overall, the majority of studies had a moderate risk of bias, with Sondhi *et al*.[16] having the greatest risk through concern for confounding and outcome measurement. In contrast, Mitchell *et al*.[30] and Kleine Holthaus *et al*.[31] showed lowest overall risk. These results called for better study designs, improved data management, and greater measurement reliability to reduce bias and enhance research validity.

## DISCUSSION

Gene therapy has shown itself to be an effective therapeutic option for BD, targeting the etiology of the disease at the genetic level and thus slowing the progression of the disease. Gene therapy's capacity to produce symptom relief and slow the progress of the disease has been supported in numerous preclinical and preliminary clinical trials. AAV-mediated gene transfer has been used in most of the studies due to its capability of crossing the BBB with ease and successfully transducing neuronal cells. For instance, some studies have reported restoring enzyme activity of CLN2 and CLN5 models with enhancing neurological functions and prolonging survival in animal models by studies such as Holthaus *et al*. (2019) and Mitchell *et al*. (2023).[27,28] Holthaus *et al*. (2019) published the rescue of a mouse model of neurodegenerative CLN6 BD with neonatal brain-directed AAV gene therapy.[27] Conversely, Mitchell *et al*. (2023) said that long-term safety and dose escalation support intracerebroventricular delivery of CLN5 gene therapy in sheep, which translated to clinical application.[28] More importantly, Sondhi *et al*. (2020) demonstrated direct brain parenchymal administration of AAV-rh. 10 expressing CLN2 substantially slowed down human disease progression during clinical trials.[16]

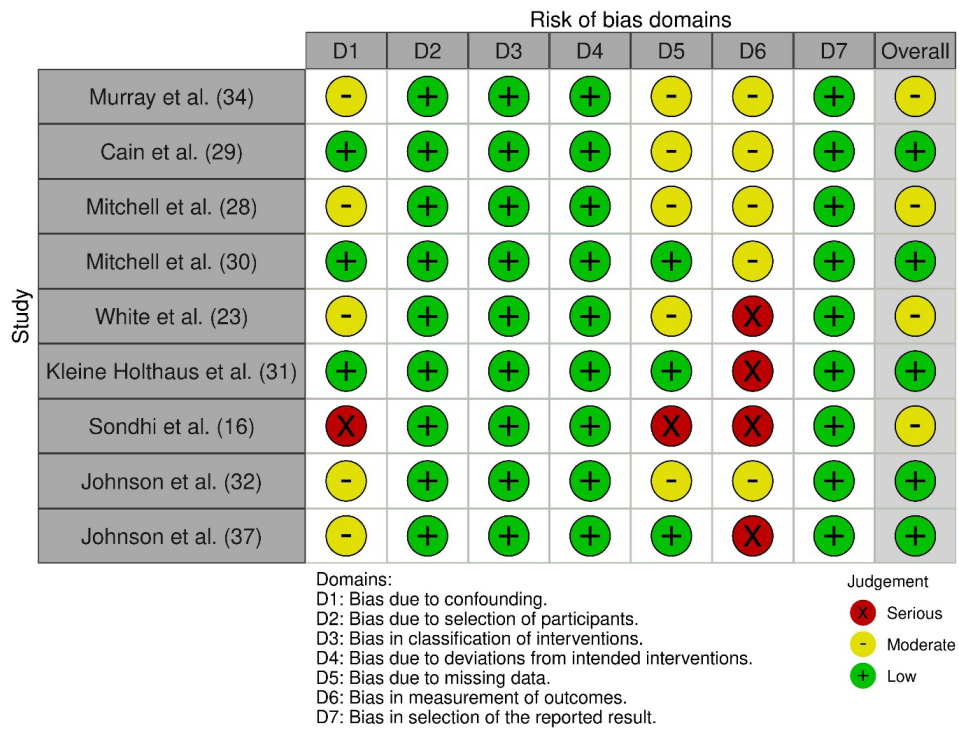


Figure 4. Bias assessment using the ROBINS-I tool

Deliverability varies, effectiveness is different, and side effect profiles are diverse. Holthaus *et al*. (2019) and Cain *et al*. (2019) focused on systemic and neonatal brain-directed gene therapy[27,29], studies like Sondhi *et al*. (2020) and Murray *et al*. (2023) used intracerebroventricular delivery, which showed varied efficacy in the distribution of genes.[16,34] Most studies reported minor side effects; however, the long-term safety of intracerebroventricular CLN5 therapy is emphasized by Mitchell *et al*. (2023).[28] Instead, Johnson *et al*. (2020) suggested that systemic AAV9 delivery can cause immune responses that must be further examined [32]. Therapeutic efficacy was also mixed, with Holthaus *et al*. (2019) and White *et al*. (2021) demonstrating significant retinal degeneration reductions with CLN6-targeted therapies[23,27], whereas Sondhi *et al*. (2020) reported delayed disease onset in CLN2 models.[16] Most notably, Murray *et al*. (2023) demonstrated improved survival and motor function after dual intracerebroventricular administration in sheep treated with CLN5.[33]

Still, a number of obstacles stand between this present stage of discovery and an efficacious, safe application of gene therapy to the BD patient population. The biggest hurdle is genetic heterogeneity, as BD encompasses a number of different genetic subtypes, CLN1-CLN14, which require targeted therapeutic approaches. Although AAV-based gene therapy has been effective for some of the subtypes, such as CLN2 and CLN5, it is still not clear whether this approach will work for all types. This makes it hard to have a single strategy for gene therapy that is generalized. The transmission of therapeutic genes to the CNS is not an easy task owing to the restrictive function of the BBB. Although AAV9 has shown some capacities for crossing the BBB, it is still difficult to achieve widespread neuronal transduction. Other alternate delivery systems have been examined by Johnson *et al*. (2023) explored various gene delivery strategies, including intrathecal, intraventricular, intraparenchymal, and systemic administration, finding that CNS-wide distribution was most effective with intrathecal and intraventricular routes, while systemic delivery required higher doses and intraparenchymal injections remained highly invasive.[37] In most gene therapies, host immunity to viral vectors could be the bottleneck. Patients might produce neutralizing antibodies against AAV vectors that decrease the transduction efficiency and make repeat dosing challenging. Such strategies are immunosuppressive regimens or engineered AAV variants of low immunogenicity, which are of great interest, and of course, a study like this by Gonzalez *et al*. (2022). Early intervention would be vital to maximize gene therapy benefits, given the fact that progressive neuronal loss characterizes BD. The challenge comes with uncertainty regarding the best treatment window as a result of the variability seen in the time of onset and progression of disease in patients.[22] Some of the research studies, like Nittari *et al*. 2022 and Morison *et al*. 2025, tend to support this early diagnosis and screening approach. The long-term effects of gene therapy cannot be defined at this time. Risks involve insertional mutagenesis, off-target effects, and a slow waning of transgene expression over time.[10,26] Follow-up studies by such authors as long-term follow-up studies by authors like Reyes *et al*. (2020) and Limia *et al*. (2022) will be able to establish the long-term safety and durability of gene therapy in BD patients.[17,21]

The above problems can be rectified through research on better gene delivery techniques, optimization of vector design, and improvement of patient selection criteria. Future research areas would be the development of new vectors that express improved CNS transduction efficiency but possibly have lower immunogenicity (Gonzalez *et al*., 2022), combinatorial strategies that employ gene therapy in combination with either enzyme replacement or pharmacological treatments to achieve better therapeutic efficacy (Rodriguez-Martinez *et al*., 2023), techniques such as CRISPR-Cas9 and base-editing use for making DNA-level corrections of genetic mutations (Brudvig & Weimer, 2022), advancements in genomics as well as biomarker studies to make therapeutic approaches more patient-specific (Schulz *et al*., 2024).[14,15,20,22]

Although AAV9 is commonly employed for CNS gene therapy because of its capacity to penetrate the BBB, other AAV serotypes can provide unique strengths based on transduction efficiency, immune response, and biodistribution. Comparative studies have investigated alternative vectors including AAVrh.10, AAV2, and AAV5, with different features for CNS purposes (Kaplitt *et al*., 2007; Mueller *et al*., 2020; Marks *et al*., 2010).[40,38,39]

AAVrh.10, an engineered vector, has been investigated for neurodegenerative diseases due to enhanced CNS penetration and widespread transgene expression in neurons and glia. Preclinical models for Batten disease demonstrate AAVrh.10 to offer wider CNS distribution than

**Comparison of AAV9 vs. Other AAV Serotypes**

| AAV vector | BBB penetration | Primary CNS targets | Immune response | Clinical use |
|---|---|---|---|---|
| **AAV9** | Strong | Neurons, astrocytes | Mild (pre-existing NAbs possible) | FDA-approved for SMA (*Zolgensma*)[38] |
| **AAVrh.10** | Moderate | Widespread CNS tropism | Lower than AAV9 | Used in preclinical ALS and Batten disease studies[40] |
| **AAV2** | Poor | Neurons (localized transduction in striatum) | Higher immune response than AAV9 | FDA-approved for RPE65 blindness (*Luxturna*)[39] |
| **AAV5** | Limited | Astrocytes, oligodendrocytes | Lower than AAV2, but still immunogenic | Being explored for Parkinson's disease[42] |

AAV9, with less immune response (Mueller *et al*., 2020).[38]

AAV2, due to its strong neuronal tropism, has so far been confined largely to focal intraparenchymal injections because of its failure to cross the BBB. Focal benefit was proved in Parkinson's disease studies (Kaplitt *et al*., 2007), but systemic delivery is a problem because it causes immune stimulation. For BD, AAV9 remains the most widely explored vector, but AAVrh.10 and hybrid vectors may offer advantages in reducing immune responses and improving transduction efficiency. Future clinical trials should assess whether alternative vectors or engineered capsid variants improve outcomes in patients with NCL disorders.[40]

*Early diagnosis and NCL subtypes: Timing of intervention*

The neuronal ceroid lipofuscinoses (NCLs) represent a spectrum of disorders that vary in age of onset, progression rate, and clinical severity. Early intervention with gene therapy is critical, as neuronal loss begins before overt symptoms appear in many NCL subtypes (Bharucha-Goebel *et al*., 2024).[41]

For infantile and late infantile forms (CLN1, CLN2, CLN5, CLN6), early intervention is crucial as neurodegeneration is rapid. Gene therapy trials for CLN2 using AAV-TPP1 delivery have demonstrated functional improvements when treatment is initiated early, reinforcing the need for early genetic screening (Bharucha-Goebel *et al*., 2024).

In juvenile-onset forms such as CLN3, the disease progresses more gradually, allowing a wider treatment window. However, studies suggest that intervening before severe neurodegeneration begins may optimize long-term therapeutic benefits.[41]

| NCL subtype | Gene | Onset age | Key symptoms | Progression rate | Potential benefit from early gene therapy |
|---|---|---|---|---|---|
| **CLN1 (Infantile NCL)** | *PPT1* | <2 years | Seizures, psychomotor delay | Rapid | Very High (early neuronal loss)[41] |
| **CLN2 (Late Infantile NCL)** | *TPP1* | 2-4 years | Language regression, motor decline | Moderate to Rapid | High (enzyme deficiency-based onset)[38] |
| **CLN3 (Juvenile NCL)** | *CLN3* | 4-7 years | Vision loss, cognitive impairment | Slow | Moderate (gene therapy may stabilize progression)[38] |
| **CLN5/CLN6 (Late Infantile to Juvenile NCL)** | *CLN5*/*CLN6* | 2-6 years | Ataxia, seizures | Variable | High (pre-symptomatic treatment may delay onset)[40,42] |

*Diagnostic challenges in NCLs*

Symptoms of NCLs often mimic conditions such as epilepsy, autism spectrum disorders, or retinal dystrophies, leading to misdiagnosis and delays in treatment (Bharucha-Goebel *et al*., 2024).[41] Unlike spinal muscular atrophy (SMA) or lysosomal storage disorders, NCLs are not routinely included in newborn screening programs, which limits early detection (Mueller *et al*., 2020).[38] The age of onset and disease severity vary significantly among NCL subtypes, making it challenging to define an optimal treatment window for gene therapy (Marks *et al*., 2010).[39]

Expanding genetic screening programs, developing biomarker-based early detection, and utilizing advanced retinal imaging techniques could significantly improve early diagnosis and facilitate timely gene therapy interventions (Kaplitt *et al*., 2007; Schultz *et al*., 2018).

*Immune responses to AAV9 in CNS gene therapy*

One of the major limitations of AAV-based gene therapy is the host immune response to the viral capsid, which can compromise vector efficacy and safety. Mueller *et al*. (2020) reported that intrathecal AAV administration for ALS patients led to meningoradiculitis, a severe inflammatory reaction involving nerve roots and the meninges.[38] Similarly, in a clinical trial on Giant Axonal Neuropathy, Bharucha-Goebel *et al*. (2024) found that AAV9 administration triggered immune-related complications, necessitating immunosuppressive regimens such as corticosteroids, tacrolimus, and rapamycin to mitigate T-cell activation and neutralizing antibody formation. These findings underscore the importance of immune modulation strategies when designing AAV9-directed therapies for BD.[41]

Apart from cell-mediated immunity, pre-existing anti-AAV antibodies pose another hurdle. Kaplitt *et al*. (2007) demonstrated that AAV gene therapy in patients with Parkinson's disease was significantly impacted by the level of neutralizing antibodies, which reduced long-term transgene expression. This suggests that pre-treatment AAV antibody screening and potential immunosuppressive protocols will be needed to ensure maximum treatment success.[40]

*Challenges in AAV9 distribution within the cns*

Although AAV9 is able to cross the BBB, uniform transduction of prominent neuronal populations is a challenging task. Marks *et al*. (2010) studied AAV2-neurturin treatment for Parkinson's disease and found that suboptimal vector distribution limited its therapeutic potential.[39] Alternatively, Mueller *et al*. (2020) demonstrated that while AAV-miR-SOD1 was present within the spinal cord, it did not significantly diminish SOD1 levels in CSF, suggestive of inadequate diffusion upon intrathecal delivery. This highlights the need to optimise delivery methods since traversing the BBB is not necessarily the same as optimal gene transduction in the CNS.[38]

To reverse this, other routes of delivery have been explored. Kaplitt *et al*. (2007) demonstrated that direct intraparenchymal injection of AAV into the subthalamic nucleus produced localized but effective transduction, and targeting strategies may be more appropriate for diffuse neurodegenerative diseases, such as Batten disease. Intrathecal, ICV, or direct intracerebral injections could be used in future work to assess if these would be more effective distribution in BD models.[40]

*Potential toxicity and long-term safety issues*

Though promising, AAV gene therapy is not completely risk-free. Mueller *et al*. (2020) reported elevated liver enzymes, which suggested potential hepatic toxicity, while Bharucha-Goebel *et al*. (2024) reported neuropathy and adverse immune reactions following high-dose AAV9 treatment.[38,41] In addition, long-term follow-up from Parkinson's disease trials (Marks *et al*., 2010) revealed that tumours formed in a few patients, which underscores the need for long-term safety monitoring.[39]

*Combination therapy: Carbenoxolone as a promising adjunct to CLN3 disease*

Early findings suggest that combination therapies can potentially enhance the efficacy of gene therapy for Batten disease. An inhibitor of GAP junctions, carbenoxolone (CBX), has been discovered to restore lipid membrane fluidity defects in cells lacking CLN3 and regain salient pathological features of the disease (Schultz *et al*., 2018). Because CLN3 mutations disrupt lysosome function and thwart endosomal-lysosomal transport, the membrane-stabilizing property of CBX could have an additional therapeutic effect when combined with gene therapy.[42]

Schultz *et al*. (2018) demonstrated that CBX rectifies several significant abnormalities in CLN3-deficient cells and mouse models efficiently. Restoration of lipid microdomain stability is one of its notable effects, which

normalizes cholesterol distribution and relieves endocytic defects in endothelial cells of Cln3-/- mice, thereby correcting membrane fluidity disturbances. In addition, CBX has been shown to improve BBB integrity by restoring astrocyte endfeet morphology, typically defective in Batten disease. Another significant observation is that CBX treatment results in the decrease of lysosomal autofluorescent storage material, a characteristic of disease progression, demonstrating its capacity to inhibit pathological accumulation in affected cells. These observations indicate that CBX could be a valuable adjunct therapy for Batten disease, especially when combined with gene therapy.

These findings suggest that CBX is able to reverse secondary cellular dysfunctions that persist in the face of CLN3 gene replacement therapy. Since BD features diffuse neurodegeneration and disruption of BBB, combining gene therapy with CBX may provide both genetic correction and stabilization of membranes for the purpose of promoting improved neuronal survival and function.[42]

Future research should investigate whether co-administration of CBX with AAV-mediated gene therapy enhances long-term therapeutic effects. Additional research on other small molecules that mimic the effects of CBX on membrane stability and lipid microdomains may further enhance combination treatment protocols for CLN3 and other forms of Batten disease.

There are limitations to this review. BD has multiple subtypes (CLN1-CLN14) that require special treatment, while AAV-mediated treatment has been successful only in CLN2 and CLN5 to a large extent. Gene therapy in the CNS is refractory to the BBB, where limited transduction of the neurons by AAV9 rules out various methods to attain the result. Immunity that includes neutralizing antibodies and T cell activation also shortens the efficacy of gene therapy and complicates multiple dosing. Variability in disease onset makes the timing of optimal treatment challenging, which emphasizes the importance of early diagnosis. Safety concerns for long-term effects, such as insertional mutagenesis and transgene expression decline, require long-term follow-up. AAV9 is most frequently used as a vector, yet others such as AAVrh.10 and AAV5 may be superior at transduction with reduced immunogenicity but require further data.

Subsequent gene therapy studies for Batten disease will seek to optimize gene delivery through the use of new vectors with enhanced blood-brain barrier crossing and CNS targeting ability, for example, engineered AAV strains or lipid nanoparticle-vectors. Combination treatments, such as gene therapy combined with enzyme replacement or with low-molecular-weight drugs like carbenoxolone, potentially enhance therapeutic benefits. Precision medicine approaches based on genomics advances and biomarker discovery potentially allow treatment regimens tailored to the individual patient. Gene-editing tools like CRISPR-Cas9 and base-editing can potentially repair genetic mutations at the DNA level. Expansion of genes added to panels of genetic testing and development of biomarker-guided screening tests can potentially enable early screening and diagnosis and even enable timely interventions.

To overcome immunobarricades, research on immunosuppressive treatment regimens and constructed low-immunogenicity AAV vectors can potentially decrease host immune reactions and increase tolerability under repeated dosing. Safety monitoring of long-term effects with long-term follow-up studies should also be performed to assess the persistence, safety, and potential late-onset side effects of gene therapy in BD patients.

In conclusion, gene therapy represents a potentially effective treatment option for BD by targeting its genetic aetiology. Promising preclinical and early clinical trial results, especially with AAV-mediated therapies, enhance enzymatic activity and reduce neurodegeneration. There are still hurdles to overcome, such as immune reactions, limitations of the blood-brain barrier, and heterogeneity of treatment effectiveness. Further research must optimize vector design, delivery routes, and combination therapy and prioritizing early diagnosis and long-term safety. In spite of these challenges, gene therapy has great promise to enhance the prognosis for patients with BD.

## REFERENCES


1. Rechtzigel M, Meyerink B, Leppert H, *et al*. Transmembrane batten disease proteins interact with a shared network of vesicle sorting proteins, impacting their synaptic enrichment. *Front Neurosci* 2022; 16:834780. https://doi.org/10.3389/fnins.2022.834780
2. Basak I, Hansen R, Ward M, Hughes S. Deficiency of the lysosomal protein cln5 alters lysosomal function and movement. *Biomolecules* 2021;11(10): 1412. https://doi.org/10.3390/biom11101412
3. Feuerborn M, Keirns C, Barohn R. Building a bridge for batten disease. *RRNMF Neuromuscul J* 2021; 2(4). https://doi.org/10.17161/rrnmf.v2i4.1535
4. Minnis C, Thornton C, FitzPatrick L, McKay T.

Cellular models of batten disease. *Biochim Biophys Acta Mol Basis Dis* 2020; 1866(9):165559. https://doi.org/10.1016/j.bbadis.2019.165559
5. Reddy S, Brahmbhatt H. Application of anticonvulsants, antiepileptic drugs, and vitamin c in the treatment and analysis of batten disease. *Cureus* 2022;14(1):e21745. https://doi.org/10.7759/cureus.21745
6. Dev N, Kumar R, Sharma S, Sharma M. Batten's disease: A seizure disorder's battle for diagnosis. *Ann Indian Acad Neurol* 2021; 24(6): 953-5. https://doi.org/10.4103/aian.aian_769_20
7. Huber RJ. Altered protein secretion in batten disease. *Dis Model Mech* 2021;14(12): dmm049152. https://doi.org/10.1242/dmm.049152
8. Yap S, Mathavarajah S, Huber R. The converging roles of batten disease proteins in neurodegeneration and cancer. *iScience* 2021;24(4):102337. https://doi.org/10.1016/j.isci.2021.102337
9. Leppert H. Sortilin inhibition treats multiple neurodegenerative lysosomal storage disorders. *bioRxiv* 2023;559064. https://doi.org/10.1101/2023.09.22.559064
10. Nittari G, Tomassoni D, Roy P, Martinelli I, Tayebati S, Amenta F. Batten disease through different in vivo and in vitro models: a review. *J Neurosci Res* 2022;101(3):298-315. https://doi.org/10.1002/jnr.25147
11. Huber R. Recent insights into the networking of cln genes and proteins in mammalian cells. *J Neurochem* 2023;165(5):643-59. https://doi.org/10.1111/jnc.15822
12. Fyke Z. Reduction of neuroinflammation and seizures in a mouse model of cln1 batten disease using the small molecule enzyme mimetic, n-tert-butyl hydroxylamine. *bioRxiv* 2024. https://doi.org/10.1101/2024.05.01.591955
13. Østergaard J. Gait phenotype in batten disease: a marker of disease progression. *Eur J Paed Neurol* 2021;35:1-7. https://doi.org/10.1016/j.ejpn.2021.09.004
14. Rodriguez-Martinez A. Intravitreal enzyme replacement for inherited retinal diseases. *Curr Opin Ophthalmol* 2023;35(3):232-7. https://doi.org/10.1097/icu.0000000000001029
15. Brudvig J, Weimer J. Cln7 gene therapy: hope for an ultra-rare condition. *J Clin Invest* 2022;132(5):e157820. https://doi.org/10.1172/jci157820
16. Sondhi D, Kaminsky S, Hackett N, *et al*. Slowing late infantile batten disease by direct brain parenchymal administration of a rh.10 adeno-associated virus expressing cln2. *Sci Transl Med* 2020;12(572):eabb5413. https://doi.org/10.1126/scitranslmed.abb5413
17. Reyes E, Lehwald L, Augustine E, *et al*. Intracerebroventricular cerliponase alfa for neuronal ceroid lipofuscinosis type 2 disease: clinical practice considerations from us clinics. *Pediatr Neurol* 2020;110:64-70. https://doi.org/10.1016/j.pediatrneurol.2020.04.018
18. Wright G, Georgiou M, Robson A, *et al*. Juvenile batten disease (cln3): detailed ocular phenotype, novel observations, delayed diagnosis, masquerades, and prospects for therapy. *Ophthalmol Retina* 2020; 4(4):433-45. https://doi.org/10.1016/j.oret.2019.11.005
19. Chen X, Dong T, Hu Y, *et al*. Aav9/mfsd8 gene therapy is effective in preclinical models of neuronal ceroid lipofuscinosis type 7 disease. *J Clin Invest* 2022;132(5). https://doi.org/10.1172/jci146286
20. Schulz A. The parent and family impact of cln3 disease: an observational survey-based study. *Orphanet J Rare Dis* 2024;19(1). https://doi.org/10.1186/s13023-024-03119-8
21. Limia C, Baird M, Schwartz M, Saxena S, Meyer K, Wein N. Emerging perspectives on gene therapy delivery for neurodegenerative and neuromuscular disorders. *J Pers Med* 2022;12(12):1979. https://doi.org/10.3390/jpm12121979
22. Gonzalez T, Simon K, Blondel L, *et al*. Cross-species evolution of a highly potent aav variant for therapeutic gene transfer and genome editing. *Nat Commun* 2022; 13(1):5947. https://doi.org/10.1038/s41467-022-33745-4
23. White K, Nelvagal H, Poole T, *et al*. Intracranial delivery of aav9 gene therapy partially prevents retinal degeneration and visual deficits in cln6-batten disease mice. *Mol Ther Methods Clin Dev* 2021;20:497-507. https://doi.org/10.1016/j.omtm.2020.12.014
24. Østergaard J, Nelvagal H, Cooper J. Top-down and bottom-up propagation of disease in the neuronal ceroid lipofuscinoses. *Front Neurol* 2022;13. https://doi.org/10.3389/fneur.2022.1061363
25. Kofoed R. Focused ultrasound increases gene delivery to deep brain structure following the administration of a recombinant adeno-associated virus in the cerebrospinal fluid. *bioRxiv* 2024. https://doi.org/10.1101/2024.02.09.579587
26. Morison L. Speech, language and non-verbal communication in cln2 and cln3 batten disease. *J Inherit Metab Dis* 2025;48(1):e12838. https://doi.org/10.1002/jimd.12838
27. Holthaus SK, Martin-Herranz S, Massaro G, *et al*. Neonatal brain-directed gene therapy rescues a mouse model of neurodegenerative CLN6 Batten disease. *Hum Mol Genet* 2019; 28(23):3867-79. doi: 10.1093/hmg/ddz210.
28. Mitchell NL, Murray SJ, Wellby MP, *et al*. Long-term safety and dose escalation of intracerebroventricular CLN5 gene therapy in sheep supports clinical translation for CLN5 Batten disease. *Front Genet* 2023;14:1212228. doi: 10.3389/fgene.2023.1212228.
29. Cain JT, Likhite S, White KA, *et al*. Gene therapy corrects brain and behavioral pathologies in CLN6-Batten disease. *Mol Ther* 2019; 27:1836-47. doi: 10.1016/j.ymthe.2019.06.015
30. Mitchell NL, Russell KN, Wellby MP, *et al*. Longitudinal in vivo monitoring of the CNS demonstrates the efficacy of gene therapy in a sheep model of CLN5 Batten disease. *Mol Ther* 2018;26(10):2366-78. doi: 10.1016/j.ymthe.2018.07.015.
31. kleine Holthaus S, Aristorena M, Maswood RN, *et al*. Gene therapy targeting the inner retina rescues the retinal phenotype in a mouse model of CLN3 Batten

disease. *Hum Gene Ther* 2020;31(13-14):709-18. doi: 10.1089/hum.2020.038.
32. Johnson TB, White KA, Brudvig JJ, *et al*. AAV9 gene therapy restores lifespan and treats pathological and behavioral abnormalities in a mouse model of CLN8-Batten disease. *Mol Ther* 2021;29(1):162-75. doi: 10.1016/j.ymthe.2020.09.033.
33. Murray SJ, Wellby MP, Barrell GK, *et al*. Efficacy of dual intracerebroventricular and intravitreal CLN5 gene therapy in sheep prompts the first clinical trial to treat CLN5 Batten disease. *Front Pharmacol* 2023:14:1212235. doi: 10.3389/fphar.2023.1212235.
34. Murray SJ, Russell KN, Melzer TR, *et al*. Intravitreal gene therapy protects against retinal dysfunction and degeneration in sheep with CLN5 Batten disease. *Exp Eye Res* 2021:207:108600. doi: 10.1016/j.exer.2021.108600.
35. Katz ML, Tecedor L, Chen YH, *et al*. AAV gene transfer delays disease onset in a TPP1-deficient canine model of the late infantile form of Batten disease. *Sci Transl Med* 2015; 7(313):313ra180. doi: 10.1126/scitranslmed.aac6191.
36. kleine Holthaus S, Smith A, Mole SE, Ali RR. Gene therapy approaches to treat the neurodegeneration and visual failure in neuronal ceroid lipofuscinoses. *Adv Exp Med Biol* 2018;1074:91-9. doi: 10.1007/978-3-319-75402-4_12.
37. Johnson TB, Brudvig J, Likhite S, *et al*. Early postnatal administration of an AAV9 gene therapy is safe and efficacious in CLN3 disease. *Front Genet* 2023:14:1118649. doi: 10.3389/fgene.2023.1118649.
38. Mueller C, Berry JD, McKenna-Yasek DM, *et al*. SOD1 suppression with adeno-associated virus and microRNA in familial ALS. *N Eng J Med* 2020;383(2):151-8. doi: 10.1056/NEJMoa2005056.
39. Marks WJ, Bartus RT, Siffert J, *et al*. Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial. *Lancet Neurol* 2010;9(12):1164-72. doi: 10.1016/S1474-4422(10)70254-4.
40. Kaplitt MG, Feigin A, Tang C, *et al*. Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. *Lancet* 2007;369(9579):2097-105. doi: 10.1016/S0140-6736(07)60982-9.
41. Bharucha-Goebel DX, Todd JJ, Saade D, *et al*. Intrathecal gene therapy for giant axonal neuropathy. *N Eng J Med* 2024;390(12):1092-104. doi: 10.1056/NEJMoa2307952.
42. Schultz ML, Tecedor L, Lysenko E, Ramachandran S, Stein CS, Davidson BL. Modulating membrane fluidity corrects Batten disease phenotypes in vitro and in vivo. *Neurobiol Dis* 2018;115:182-93. https://doi.org/10.1016/j.nbd.2018.04.010.