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Neuronal ceroid lipofuscinosis: a multidisciplinary update

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Neuronal ceroid lipofuscinosis: a multidisciplinary update

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Editorial: Neuronal ceroid lipofuscinosis: A multidisciplinary update

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Editorial on the Research Topic
Neuronal ceroid lipofuscinosis: A multidisciplinary update

Eleven papers and fifty-one authors from seven countries have contributed to the Research Topic *Neuronal Ceroid Lipofuscinosis: a Multidisciplinary Update*. Both clinical and research issues have been addressed in this collection of articles. The first paper provides a broad introduction and subsequent articles cover epidemiology and genetics, diagnosis, natural history studies, treatment and ethical implications of novel therapies, cardiac involvement in the later stages of disease and the underlying pathological mechanisms.

The state-of-art in the field of childhood NCLs was described from a number of perspectives in the first review paper of the series (Simonati and Williams). Following a brief historical survey, a clinically-oriented approach was used to describe how the early symptoms and signs represent topographical signatures of the underlying brain dysfunction and may provide clues helping clinicians to reach a conclusive NCL diagnosis rapidly. The paper goes on to document advances in NCL research and the contributions of different experimental models to enhance knowledge of the pathogenic mechanisms underlying cellular pathology in this group of diseases. Lastly, translation of experimental data into novel therapeutic approaches and the importance of symptomatic treatments, which remain the main available therapeutic approaches, were outlined.

The world-wide distribution of NCL was emphasized by the retrospective epidemiological study from South America and the Caribbean region, in which CLN2, CLN6, and CLN3 disorders were identified as the most common NCL types in those regions (Guelbert et al.). The authors have stressed that synergy between health providers, parent support organizations and the pharmaceutical industry have accelerated the use of modern diagnostic procedures.

The significance of the advances in genetic studies in NCL was discussed in the review article by Gardner and Mole which focuses on the genetic basis of phenotypic

heterogeneity (Gardner and Mole). Since the discovery of the first causative genes, more than 530 mutations have been identified across 13 NCL genes. Increasing numbers of variant disease phenotypes are being described. Identification of phenotypic heterogeneity combined with the genetic background of each patient is necessary in order to facilitate individually tailored precision medicine in order to modify disease progression in the approaching genomic medicine era.

Based on their own direct experience, which led FDA and EMA Regulatory Agencies to consider natural-history controls valid for the evaluation of efficacy in experimental therapies for CLN2 disease, Nickel and Schulz discuss the importance of collecting natural history data in clinical settings for different purposes, including to advance drug development. The most important requirements of a valid natural history disease registry compliant with data protection and sharing policies, are described. The process of providing high quality quantitative natural history data in a cohort of longitudinally assessed CLN2 disease patients is reported.

The focus of a mini review by Bartsch and Storch is the deterioration and loss of vision caused by progressive retinal degeneration. The therapeutic benefits of treating retinal dystrophies with gene-based approaches (CLN3 and CLN6 mouse models and CLN5 sheep model) and with ocular enzyme replacement therapies (CLN2 and CLN10 mouse models), has led to a clinical trial enrolling CLN2 patients to test the efficacy of intravitreal ERT. The long-term effects of these therapeutic interventions remain to be evaluated.

Ethical issues in care and treatment are the topic of a paper which reflects the long personal clinical experience in the field of this author (Kohlschütter). He identifies two main topics, the first relates to the care of individual patients affected with dementia at a young age, the use of life-prolonging measures and the planning for the end of life, the second refers to new experimental treatments and the awareness that such approaches carry the risk of prolonged survival with poor quality of life. The paper gives examples experienced by the author which offer insights for the "critical thinking" of readers. The issues encountered in caring for patients affected by NCL, but may well be common to other rare neurodegenerative diseases of childhood.

The importance of neurophysiological tools to describe disease evolution and supporting early diagnosis of NCL patients was reviewed through a careful analysis of their characteristics in several NCL types (Trivisano et al.). Authors outline how EEG and (to a less extent) evoked potentials can promptclinicians to obtain a molecular diagnosis in the early phase of any NCL form, which will help to direct patients to appropriate targeted treatments (when available) efficiently.

Reaching an early diagnosis was the aim of a nationwide screening project in Spain amongst children whose early clinical features were consistent with CLN2 (Rodrigues et al.). It used an enzymatic assay of TPP1 activity in dry blood spots, carried out through pediatricians. Authors describe the test as easy to perform, inexpensive and reliable and conclude that such a test may contribute to early delivery of ERT in this condition.

The next two articles concern cardiac involvement in CLN3 disease. In their report describing a case series of six, Handrup et al. state that pacemaker implantation is safe and positively impacts on quality of life of patients because of the presence of early cardiac conduction disorders and later left ventricular hypertrophy. The ethical implications of such therapeutic option are also commented upon. In a single case report the accuracy of comprehensive cardiac MRI findings are reported in a CLN3 disease patient. Authors recommend the advantages of cardiac MRI both for early diagnosis of cardiac complications of NCL and its value in monitoring the effects of emerging CLN3 therapies on the myocardium (Todiere et al.).

In the only review article related to experimental models of NCLs, Takahashi et al., describe the current knowledge on the role of the different glia components (astrocytes, microglia, oligodendrocytes) in brain homeostasis. They go on to focus on the most up-to-date understanding of glial pathologies and their contribution to the pathogenesis of NCLs: they highlight some of the associated challenges that require further research as obtained by their studies using genetically modified mouse models. The emerging evidence of glial dysfunction questions the traditional "neuron-centric" view of NCLs, and would suggest that directly targeting glia in addition to neurons could lead to better therapeutic outcomes.

In summary, this series of articles is drawn from world experts in NCL. It brings together basic science and new clinical knowledge, while considering the ethical implications of recent progress on individual patients, families and their physicians and clinicians.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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MINI REVIEW
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Ethical Issues in Care and Treatment of Neuronal Ceroid Lipofuscinoses (NCL)–A Personal View

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The management of Neuronal Ceroid Lipofuscinoses (NCL), a group of genetic neurodegenerative disorders mainly affecting brain and retinas, raises difficult questions for physicians and other professionals in research, pharmaceutical industry, and public health. Ethical problems in medicine cannot be solved by rational deliberation or by following formal rules. Two topics of ethical issues in the field of NCL are presented here. One group relates to the care of individual patients and centers on a life with dementia at a young age. Advanced care planning for the end of life and the use of life-prolonging measures require challenging assumptions in the best interest of a patient. A second group of questions relates to new treatments. Impressive novel putative causal therapies, such as enzyme replacement for CLN2 disease, may be only disease-modifying and carry the risk of changing a deadly disease of short duration into one with prolonged survival and poor quality of life. The wish for better therapeutic interventions in life-limiting diseases has to take such risks, but more experience is needed before definite conclusions can be drawn. The appropriateness of presymptomatic screening for a severe disease, e.g., must be carefully evaluated to avoid the disastrous experience made with the rash start of newborn screening for Krabbe disease. The ethical issues described and commented in the article reflect the personal experience of a pediatrician who has studied clinical and research questions in NCL for four decades. They should alert various professionals to the necessity of taking their own decisions in situations that are caused by rare progressive brain diseases of young persons, as typified by the NCL.

Keywords: dementia, genetic, lysosomal storage disease, children, palliative medicine, disease-modifying therapy, newborn screening

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INTRODUCTION

Ethical problems in medicine are questions that cannot be answered rationally or by following standardized rules. Answers have to evolve on the basis of a personal attempt to come to terms with an ethical dilemma. Neuronal Ceroid Lipofuscinoses (NCL), a group of genetic life-limiting neurodegenerative disorders predominantly affecting the brain and retina of children, raise questions that concern physicians involved in patient care as well as other professionals in research, pharmaceutical industry, and public health.

This review reflects the personal experience of a pediatrician who has been dealing with NCL families and research for four decades. Ethical issues encountered are presented in two sections: questions related to the care of patients and more general questions with impact on other areas

of healthcare. The article should allow readers to recognize responsibilities of various professionals taking care of NCL and show ways along which decisions can be reached.

ASPECTS OF A LIFE WITH DEMENTIA IN THE YOUNG

The different NCL, named after their defects of the genes *CLN1* to *CLN14* (1), display a high clinical variability and can manifest from birth to young adulthood (2). All forms of NCL lead to dementia, helplessness and premature death. To understand the personality and the psychosocial situation of a NCL patient, one has to realize that the individual had been healthy and developed normally before the onset of symptoms (with the exception of congenital cases), that the clinical deterioration is inexorably progressive, and that the patient and the family are repeatedly confronted with terrifying and demanding situations.

The authorization of a physician to decide on ambiguous ethical questions is derived from a thorough understanding of a patient's condition and from respecting the individuality of the situation. Apart from these prerequisites and consideration of the general principles of medical ethics [beneficence, non-maleficence, autonomy, and justice (3)], little else is needed for ethical deliberations of a responsibly acting physician.

Balancing Patient Autonomy and Dementia

A demented person unable to communicate verbally may still be able to express a judgement on his or her own situation. The story of a young man told in **Box 1** illustrates this poignantly.

Comment. The ethical problem was deciding whether an indication existed for starting artificial nutrition, an invasive intervention that must have a treatment goal, must be supported by scientific evidence, and requires patient consent (4). In this case, indisputable evidence was absent, the patient's will could only be guessed from non-verbal signals, and a decision had to be made together with parents on the basis of the personal judgment of a physician familiar with the patient's situation ("shared decision making") (5). In some countries, it is not legal to withdraw artificial nutrition in terminally ill patients as it is not considered a medical intervention but a component of basic care (4). In these countries, it might be even more difficult to reach a balanced decision.

*"Consensus with the nursing team": For problems as described in **Box 1**, multidisciplinary discussion and shared decision making is usually advised. Consensus of the multiprofessional team is of great importance for sustained patient care. While various involved healthcare professionals may have different helpful arguments and should be heard, the final responsibility should rest on a single physician.*

Vital Decisions in the Patient's Best Interest

Choices of adults regarding the end of their lives are liberally discussed at present, while legal frameworks remain controversial (6, 7). Decision-making capacities of children and decisionally vulnerable adults are even more delicate, but must not be neglected (8, 9).

There is wide consensus that vital medical decisions should be taken in the best interest of the patient. Defining this best interest is difficult against the background of cognitive impairment and in children. Substitutes must therefore form a proper idea of a patient's best interest. In children, the natural substitutes are their parents. For them, this assignment can be a severe burden that requires great emotional support and understanding of the consequences of a decision. Supporting the parents, informing and "educating" them appropriately (which typically requires many repeated discussions) is a demanding job for the doctor in charge.

Sometimes, accepting death as a consequence of a decision may be in the best interest of the patient. A pediatrician will have to make up his own mind about the patients' wish and then try to make the parents understand what is at stake. When the physician succeeds with this, outside views coming from the family and from their environment may further complicate the situation. For a discussion of the best-interests-of-the-child framework, see Engelhardt (10) and Zawati et al. (11).

Ideally, even parents as medical lay persons can appropriately decide in difficult medical matters (see **Box 1**), provided they are adequately informed and understand the consequences, not only in respect to their child's life but also in regard to their further existence in their social world.

Advice can be given using differing attitudes, more paternalistic or more objective, depending on the intellectual

BOX 1 | A resolute personality.

Arthur (not his real name) was diagnosed with juvenile CLN3 disease as a first grader after his vision had deteriorated. He was transferred to a school for the visually handicapped. Gradually, he lost intellectual capacity and developed seizures but remained a strong boy, good-humored when he got his ways and fighting fiercely against everything he did not like. His parents acquainted themselves with the dire prognosis and concluded that, should their son become helpless, any measure to prolong life unnaturally would appear to them entirely inappropriate. They asked if I, as the familiar specialized pediatrician, would stand by them should such a situation arise. I kept seeing Arthur from time to time.

Arthur ended up helpless, unable to converse verbally and being cared for in an excellent nursing home. When he was 27, his parents, still in custody, called me because he had started, without comprehensible reason, to refuse feeding and reacted fiercely to any attempt of offering food. Multidisciplinary examination did not reveal an organic explanation. When I saw him, he was in good spirits and reacted in a friendly way. When offered a spoonful of a favorite food, he vehemently turned away his head. His parents assumed his behavior reflected that Arthur was "fed up" with life. I examined him, observed him for a long time, and collected additional information from persons who knew him. One of his teachers commented that he had always reacted in such a way when something went against his will.

In the end, I concluded that the parents' interpretation was probably right and that the arguments for starting artificial nutrition were less weighty than those for withholding it. Consensus of opinion with the nursing team was reached and feeding was discontinued. Arthur's mouth was kept moist. He remained quiet without signs of discomfort. A few days later, he developed fever and a cough, and subsequently pneumonia. When he appeared to have difficulty breathing, he was given oxygen and eventually morphine. Shortly afterwards, he died. Arthur's death certificate read "natural death."

abilities and psychological circumstances. When consensus exists between both parents and their physician, a vital decision can be made without sharing responsibility with persons outside the family. If this is not possible in exceptional cases, advice must be sought from other sides, which may include institutional ethical review boards. External advisers, however, should not give the impression that they are taking a final decision. They should rather facilitate discussion of arguments and help parents find a decision they can live with. For further discussion, see Hain (12).

Comment: Measures or interventions to be taken or not in critical situations to be expected must be discussed well in advance. At an international workshop with participation of patient families, it was concluded that decisions on life-prolonging interventions in children with advanced NCL or other degenerative brain diseases are highly individual but can be made in a rationally and emotionally acceptable way (13).

Quality of Life

In the case of a helpless, non-communicative person with an incurable life-limiting disease, speculations about the patient's quality of life are frequently expressed. From my own experience, quality of life is an umbrella term covering a wide variety of concepts. While an adult may declare that he would "rather die than end up in a wheelchair," the parent of a child in the end stage of a progressive brain disease may say that there is still sometimes a smile on the face of her child and therefore the child's quality of life is good. In many instances, the quality of life of a severely incapacitated child fully depends on the irreplaceable care, love, and health of parents. When these "quasi inexhaustible resources" are lost due to the progress of time, when a patient's "child appeal" has disappeared or the parents have died, a patient's quality of life may strongly decrease.

Comment. Ethical decisions about a patient's quality of life should be considered as highly individual, which confers low priority to "moral" considerations (moral referring to supra-individual norms) from outside.

Families' Quest for New Therapies

NCL are incurable diseases, but at present, several novel targeted therapy approaches are emerging, and most parents of an affected child express a strong desire to participate at a study "at any price." While eligibility of patients with rare diseases to participate in a clinical trial is a complex matter, families must understand some basic questions: the unpredictable outcome and risks of a newly proposed procedure in general, and the individual suitability of their child at the actual stage of the progressive disease.

Comment. Advising a family in respect to a new treatment requires much personal judgement. A study in families with metachromatic leukodystroph (a progressive neurodegenerative disease with similarities to NCL) showed that preservation of speech or active communication, as well as a stop of disease progression, had the highest parental priorities expected for new treatments (14).

ASPECTS RELATED TO NOVEL THERAPIES

Recently, clinical trials that were performed under rigidly controlled circumstances have shown that enzyme replacement therapy (ERT) in patients with CLN2 disease can significantly slow down the progressive deterioration of neurological function (15). Not surprisingly, this exciting achievement is fraught with new challenging ethical questions.

Experimental Therapies Outside Clinical Trials

Once a new treatment has been shown to be safe and efficacious and has been approved by the regulatory authorities, it remains experimental for some time for a variety of reasons. In the case of ERT for CLN2 disease, which involves repeated complex invasive procedures for drug delivery to the brain (15), one of the remaining questions is how the results will look like when the treatment is used under less strictly controlled conditions. Such observations have been made in France (16) and in Colombia (17) but do not suffice yet in this respect.

Comment. Once an experimental drug is selling well, the producing company's interest to critically follow late results of treatment may fade away. Whose obligation is it then to take over responsibility?

New Targeted Therapies—Are They Only Disease-Modifying?

Another aspect of novel treatments in severe rare diseases causes much greater concern. While enzyme replacement therapy for CLN2 disease effectively slows neurological progression, there are no long-term results yet (15). In the waiting time, we have to make efforts to shift the time point of diagnosis to younger ages, as an early start of treatment, ideally in pre-symptomatic children, will most probably have an impact on the results.

In the meantime, clinicians and researchers have to live with an uncomfortable risk. Untreated late infantile CLN2 disease leads to loss of all human abilities and death at about 10 years of age. Enzyme replacement treatment delays the losses of mental and motor function (not that of vision) significantly but with unknown psychomotor functions later in life. It may be that treatment modifies a terrible disease with short life expectancy into a long life with possibly poor quality of life (**Figure 1**).

Analogous problems have occurred with other neurodegenerative disorders of childhood. Infantile Krabbe disease is a lysosomal degenerative brain disease that leads to death within the first 2 years of life. Hematological stem cell transplantation can curb the progressive brain destruction and leads, when performed shortly after birth, to prolonged survival and achievement of more psychomotor abilities than untreated patients can obtain. The overall results of treatment, however, have been disappointing (18). Long before this became recognizable and other important facts being also unknown, newborn screening for Krabbe disease was enthusiastically introduced in the state of New York in 2006. This project has

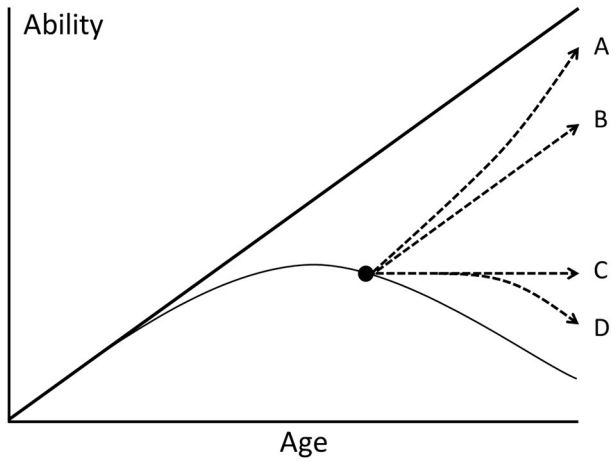

FIGURE 1 | Scheme of the psychomotor development of children. The thick straight line represents the development of a healthy child who steadily gains abilities with increasing age. The thin curved line represents a child with degenerative brain disease. When effective therapy is started after appearance of symptoms (black dot), different deviations from the natural course of disease are possible (broken lines A–D).

been associated with many false hopes and binding of enormous resources (19).

Spinal muscular atrophy (SMA), a progressive neuromuscular disorder, is caused by a gene defect leading to the dying off of motoneurons. Infants with the SMA1 type mostly die within the first 2 years of life or require ventilation >16 h per day. Recently developed genetically targeted therapies drastically alter the rapid progression of the disease in the 1st years of life; the later course of disease remains uncertain (20) as longitudinal data are outstanding. Extraordinarily high costs (drug prices, personnel resources, etc.) in the context of limited available evidence are calling for a just balance of interests of patients, healthcare systems, pharmaceutical industry, and society (21).

Comment. Clinical research has to take risks to achieve therapeutic progress but may contemporaneously create new problems by disease-modifying therapies. We must continue to analyze what we are doing and define responsibilities.

Newborn Screening

The principle of newborn screening for treatable rare diseases is detecting them in a preclinical stage and preventing the outbreak of symptoms by early intervention. Given the treatability of CLN2 patients by enzyme replacement therapy (15), the disease becomes an applicant for inclusion into routine newborn screening. Feasibility and reliability of testing for the disease in dried blood samples for use with screening have been demonstrated (22).

The "classic" view of the appropriateness of newborn screening for a disease is that it can be treated effectively. Due to the lack of long-term data on enzyme replacement therapy in CLN2 patients, this disease at present does not qualify for newborn screening. Recently, a "non-classic" view has argued that screening is appropriate even for diseases without available treatment. Proponents of this view list as values of such an expanded screening that it gives parents diagnostic and prognostic information about their child, allows them to make more informed reproductive decisions, improve symptomatic therapies, and will stimulate research on poorly understood diseases (23). Implementing an expanded screening of this kind appears at present impossible in Europe, given legal and other restrictions. Nevertheless, studies of newborn screening for several lysosomal storage disorders (24), among them for mucopolysaccharidoses (25, 26), have documented their feasibility and have investigated ethical, political, and other remaining obstacles for including these diseases into general screening programs.

Comment. A more liberal view of newborn screening that would include diseases with questionable or even absent treatability bears some attractivity. Among the obstacles of implementation are the complexity of an imperative prenatal education (27) and respect for a "right not to know" (28).

Public Health and General Research Issues

NCL belong to the thousands of diseases for which the fact of their rarity adds to suffering of patients and hampers the development of treatments. Rarity leads to insufficient knowledge of the course of disease and of disease mechanisms and to too few participants in clinical trials of proposed new therapies (29, 30). Rarity creates a host of questions for stakeholders in the field: affected patients with their families, patient organizations and supporting foundations, healthcare providers (medical services and pharmaceutical industry), insurance or other payments systems, public health policymakers, and research institutions.

Clinical research faces the impact of a set of rare disease characteristics that influence the methodology of completing robust studies (29). A study of the perspectives of different stakeholders on therapy-related research concluded that stakeholders have divergent views on rare disease research but share concerns about the risks vs. benefits of therapies when making their decisions (31).

Development of drugs or procedures for rare diseases and their pricing is causing much debate. An extreme situation was reached when an individualized drug therapy with an allele-specific oligonucleotide was developed for a single patient suffering from a type of NCL caused by a specific rare mutation of the *CLN7* gene (32). A systematic review of ethical problems linked to rare diseases and orphan drugs lists the following major issues: the funding of research, the significance of non-economic values like compassion and beneficence in decision-making, the identification of limits to labeling diseases as rare, barriers to supranational cooperation, and determining panels of decision-makers (33). Rare disease policies and reimbursement systems for orphan medicinal products and healthcare services differ greatly between countries (34, 35).

In a process known as venture philanthropy, private foundations obliged to specific diseases have formed partnerships with industry and federal agencies to share the financial risk of therapeutic development (36). For three lysosomal storage

disorders, a charitable access program for patients in underserved communities worldwide has been instituted (37). This program could become a model for cooperation between industry, patient organizations, and governmental and non-governmental organizations in fighting rare diseases.

Comment. The rarity alone of a disease causes a multitude of problems. As 3–6% of the world population are affected by thousands of rare diseases (38), the potential load required by these diseases for healthcare and research is astronomical.

Further Topics

In NCL, as in many genetic devastating diseases, professionals will be confronted with further topics requiring difficult ethical decisions: prenatal diagnosis, pre-implantation diagnosis, or results of carrier screening as determinants of reproductive choices (39). These are beyond the scope of this review.

DISCUSSION

Difficult questions related to NCL or similar diseases are manifold and have been presented here as forming two major groups. One group centers on the care of individual patients on their way to dementia, complete helplessness, and early death. Advanced care planning for the end of life and assumptions on the best interest of a patient constitutes one of the most challenging problems. A second group of problems mainly concerns experimental, targeted therapies. New therapies, even when astonishingly effective, carry the risk of being disease-modifying with a potentially undesirable outcome. The sections in the text on single problem areas are followed by short personal comments that try to mark particularly hot spots of discussion (printed in *italics*).

In conclusion, the ethical issues presented should make physicians and other professionals, including researchers and politicians, aware of having to take their own decisions in widely different situations caused by progressive brain diseases of young persons, as they typically occur in NCL.

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The author confirms being the sole contributor of this work and has approved it for publication.

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REFERENCES

1. Williams RE, Mole SE. New nomenclature and classification scheme for the neuronal ceroid lipofuscinoses. *Neurology.* (2012) 79:183–91. doi: 10.1212/WNL.0b013e31825f0547
2. Nita DA, Mole SE, Minassian BA. Neuronal ceroid lipofuscinoses. *Epileptic Disord.* (2016) 18:73–88. doi: 10.1684/epd.2016.0844
3. Beauchamp T. The 'four principles' approach to health care ethics. In Ashcroft R, Dawson A, Draper H, Mcmillan J, editors. *Principles of Health Care Ethics*. West Sussex: John Wiley and Sons, Inc (2007). p. 3–10.
4. Cardenas D. Ethical issues and dilemmas in artificial nutrition and hydration. *Clin Nutr ESPEN.* (2021) 41:23–9. doi: 10.1016/j.clnesp.2020.12.010
5. Santoro JD, Bennett M. Ethics of end of life decisions in pediatrics: a narrative review of the roles of caregivers, shared decision-making, and patient centered values. *Behav Sci (Basel).* (2018) 8:50042. doi: 10.3390/bs8050042
6. Ulrich LP. *The Patient Self-Determination Act: Meeting the Challenges in Patient Care*. Washington: Georgetown University Press (1999).
7. Botti C, Vaccari A. End-of-life decision-making and advance care directives in Italy. A report and moral appraisal of recent legal provisions. *Bioethics.* (2019) 33:842–8. doi: 10.1111/bioe.12615
8. Dalpe G, Thorogood A, Knoppers BM. A tale of two capacities: including children and decisionally vulnerable adults in biomedical research. *Front Genet.* (2019) 10:289. doi: 10.3389/fgene.2019.00289
9. Fay M, Guadarrama J, Colmenares-Roa T, Moreno-Licona I, Cruz-Martin AG, Pelaez-Ballestas I. The relationship between proxy agency and the medical decisions concerning pediatric patients in palliative care: a qualitative study. *BMC Palliat Care.* (2021) 20:27. doi: 10.1186/s12904-021-00723-4
10. Engelhardt HT. (2010). Beyond the best interests of children: four views of the family and of foundational disagreements regarding pediatric decision making. *J Med Philos.* 35, 499–517.
11. Zawati MH, Parry D, Knoppers BM. The best interests of the child and the return of results in genetic research: international comparative perspectives. *BMC Med Ethics.* (2014) 15:72. doi: 10.1186/1472-6939-15-72
12. Hain RDW. Voices of moral authority: parents, doctors and what will actually help. *J Med Ethics.* (2018) 44:458–61. doi: 10.1136/medethics-2017-104705
13. Kohlschütter A, Riga C, Crespo D, Torres JM, Penchaszadeh V, Schulz A. Ethical issues with artificial nutrition of children with degenerative brain diseases. *Biochim Biophys Acta.* (2015) 1852:1253–6. doi: 10.1016/j.bbadis.2015.03.007
14. Eichler FS, Cox TM, Crombez E, Dali CI, Kohlschutter A. Metachromatic leukodystrophy: an assessment of disease burden. *J Child Neurol.* (2016) 31:1457–63. doi: 10.1177/0883073816656401
15. Schulz A, Ajayi T, Specchio N, De Los Reyes E, Gissen P, Ballon D, et al. Study of intraventricular cerliponase alfa for CLN2 disease. *N Engl J Med.* (2018) 378:1898–907. doi: 10.1056/NEJMoa1712649
16. Estublier B, Cano A, Hoebeke C, Pichard S, Scavarda D, Desguerre I, et al. Cerliponase alfa changes the natural history of children with neuronal ceroid lipofuscinosis type 2: the first French cohort. *Eur J Paediatr Neurol.* (2021) 30:17–21. doi: 10.1016/j.ejpn.2020.12.002
17. Espitia Segura OM, Hernández Z, Mancilla NI, Naranjo RA, Tavera L. Real world effectiveness of cerliponase alfa in classical and atypical patients. A case series. *Mol Genet Metab Rep.* (2021) 27:100718. doi: 10.1016/j.ymgmr.2021.100718
18. Graf WD. Stem cell transplantation in Krabbe disease: new truths discovered and opinions change. *Neurology.* (2017) 89:1318–9. doi: 10.1212/WNL.0000000000004427
19. Ehmann P, Lantos JD. Ethical issues with testing and treatment for Krabbe disease. *Dev Med Child Neurol.* (2019) 61:1358–61. doi: 10.1111/dmcn.14258
20. Ojala KS, Reedich EJ, Didonato CJ, Meriney SD. In search of a cure: the development of therapeutics to alter the progression of spinal muscular atrophy. *Brain Sci.* (2021) 11:194. doi: 10.3390/brainsci11020194
21. Kirschner J, Butoianu N, Goemans N, Haberlova J, Kostera-Pruszczyk A, Mercuri E, et al. European ad-hoc consensus statement on gene replacement therapy for spinal muscular atrophy. *Eur J Paediatr Neurol.* (2020) 28:38–43. doi: 10.1016/j.ejpn.2020.07.001
22. Lukacs Z, Nickel M, Murko S, Nieves Cobos P, Schulz A, Santer R, et al. Validity of a rapid and simple fluorometric tripeptidyl peptidase 1 (TPP1) assay using dried blood specimens to diagnose CLN2 disease. *Clin Chim Acta.* (2019) 492:69–71. doi: 10.1016/j.cca.2019.02.010

23. Lantos JD. Dangerous and expensive screening and treatment for rare childhood diseases: the case of Krabbe disease. *Dev Disabil Res Rev.* (2011) 17:15–8. doi: 10.1002/ddrr.133
24. Peake RW, Bodamer OA. Newborn screening for lysosomal storage disorders. *J Pediatr Genet.* (2017) 6:51–60. doi: 10.1055/s-0036-1593843
25. Parini R, Broomfield A, Cleary MA, De Meirleir L, Di Rocco M, Fathalla WM, et al. International working group identifies need for newborn screening for mucopolysaccharidosis type I but states that existing hurdles must be overcome. *Acta Paediatr.* (2018) 107:2059–65. doi: 10.1111/apa.14587
26. Arunkumar N, Langan TJ, Stapleton M, Kubaski F, Mason RW, Singh R, et al. Newborn screening of mucopolysaccharidoses: past, present, and future. *J Hum Genet.* (2020) 65:557–67. doi: 10.1038/s10038-020-0744-8
27. Botkin JR, Rothwell E, Anderson RA, Rose NC, Dolan SM, Kuppermann M, et al. Prenatal education of parents about newborn screening and residual dried blood spots: a randomized clinical trial. *JAMA Pediatr.* (2016) 170:543–9. doi: 10.1001/jamapediatrics.2015.4850
28. Berkman BE, Hull SC. The "right not to know" in the genomic era: time to break from tradition? *Am J Bioeth.* (2014) 14:28–31. doi: 10.1080/15265161.2014.880313
29. Whicher D, Philbin S, Aronson N. An overview of the impact of rare disease characteristics on research methodology. *Orphanet J Rare Dis.* (2018) 13:14. doi: 10.1186/s13023-017-0755-5
30. Mayrides M, Ruiz De Castilla EM, Szelepski S. A civil society view of rare disease public policy in six Latin American countries. *Orphanet J Rare Dis.* (2020) 15:60. doi: 10.1186/s13023-020-1314-z
31. Tingley K, Coyle D, Graham ID, Chakraborty P, Wilson K, Potter BK, et al. Stakeholder perspectives on clinical research related to therapies for rare diseases: therapeutic misconception and the value of research. *Orphanet J Rare Dis.* (2021) 16:26. doi: 10.1186/s13023-020-01624-0
32. Woodcock J, Marks P. Drug regulation in the era of individualized therapies. *N Engl J Med.* (2019) 381:1678–80. doi: 10.1056/NEJMe1911295
33. Kacetl J, Maresova P, Maskuriy R, Selamat A. Ethical questions linked to rare diseases and orphan drugs - a systematic review. *Risk Manag Healthc Policy.* (2020) 13:2125–48. doi: 10.2147/RMHP.S260641
34. Czech M, Baran-Kooiker A, Atikeler K, Demirtshyan M, Gaitova K, Holownia-Voloskova M, et al. A review of rare disease policies and orphan drug reimbursement systems in 12 Eurasian countries. *Front Public Health.* (2019) 7:416. doi: 10.3389/fpubh.2019.00416
35. Stawowczyk E, Malinowski KP, Kawalec P, Bobinski R, Siwiec J, Panteli D, et al. Reimbursement status and recommendations related to orphan drugs in european countries. *Front Pharmacol.* (2019) 10:1279. doi: 10.3389/fphar.2019.01279
36. Ramsey BW, Nepom GT, Lonial S. Academic, foundation, and industry collaboration in finding new therapies. *N Engl J Med.* (2017) 376:1762–69. doi: 10.1056/NEJMra1612575
37. Mehta A, Ramaswami U, Muenzer J, Giugliani R, Ullrich K, Collin-Histed T, et al. A charitable access program for patients with lysosomal storage disorders in underserved communities worldwide. *Orphanet J Rare Dis.* (2021) 16:8. doi: 10.1186/s13023-020-01645-9
38. *EURORDIS The Voice of Rare Disease Patients in Europe* [Online] (2020). Available online at: https://www.eurordis.org (accessed March 01, 2021).
39. Sparks TN. Expanded carrier screening: counseling and considerations. *Hum Genet.* (2020) 139:1131–9. doi: 10.1007/s00439-019-02080-y

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REVIEW
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The Genetic Basis of Phenotypic Heterogeneity in the Neuronal Ceroid Lipofuscinoses

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The neuronal ceroid lipofuscinoses (NCLs) are a group of inherited neurodegenerative disorders that affect children and adults. They share some similar clinical features and the accumulation of autofluorescent storage material. Since the discovery of the first causative genes, more than 530 mutations have been identified across 13 genes in cases diagnosed with NCL. These genes encode a variety of proteins whose functions have not been fully defined; most are lysosomal enzymes, or transmembrane proteins of the lysosome or other organelles. Many mutations in these genes are associated with a typical NCL disease phenotype. However, increasing numbers of variant disease phenotypes are being described, affecting age of onset, severity or progression, and including some distinct clinical phenotypes. This data is collated by the NCL Mutation Database which allows analysis from many perspectives. This article will summarise and interpret current knowledge and understanding of their genetic basis and phenotypic heterogeneity.

Keywords: neuronal ceroid lipofuscinosis, batten disease, NCL, CLN, mutation, gene, lysosomal disease

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INTRODUCTION

The neuronal ceroid lipofuscinoses (NCL), also known as Batten disease, are a group of inherited neurodegenerative life-limiting diseases that share some common clinical features including epileptic seizures, progressive psychomotor decline, and visual failure, and the accumulation of autofluorescent storage material. NCL usually begins in childhood, and most are inherited in an autosomal recessive manner. More than a dozen genes have been linked to families diagnosed with NCL (**Table 1**) (1). It is likely that most genes causing NCL have been identified.

This article summarises the genetic basis of NCL and discusses correlations with disease phenotype. All mutation details can be found in the freely accessible NCL mutation database (www.ucl.ac.uk/ncl-disease).

HISTORICAL PERSPECTIVE

The concept of NCL as a group of inherited diseases first emerged in the 1960s (2), leading to classification into four broad ages of onset: infantile, late infantile, juvenile, and adult. At this time it was assumed that each of these types was caused by mutations in a different gene, named in advance as *CLN1*, *CLN2*, *CLN3,* and *CLN4*, respectively. The first genes to be identified, in the 1990s (*CLN1*, *CLN2*, *CLN3*), were responsible for the most common paediatric types. However, these first identified genes were not responsible for all childhood onset cases. For example, the genes *CLN5*,

TABLE 1 | Summary of genotype and phenotype in NCL.

Gene	Disease name(s)	Protein and location	Number of reported mutations	Widespread common mutations	Regional-specific mutations	Genotype-phenotype correlation	Age of onset	Presenting and typical clinical features
CLN1 *PPT1*	CLN1 disease	Palmitoyl protein thioesterase 1; lysosome	71	c.364A > T/p.(Arg122Trp); c.451C > T/p.(Arg151*)	c.223A > C/p.(Thr75Pro); c.29T > A/p.(Leu10*) in Scotland	**Infantile** Late infantile Juvenile Adult	**6–18 mo** 2–4 y 5–10 y >20 y	Speech and motor development delay, Rett-like hand movements, loss of developmental gains
CLN2 *TPP1*	CLN2 disease	tripeptidyl peptidase 1; lysosome	155	c.509-1G > C/p.(Val170Glyfs*29); c.622C > T/p.(Arg208*)	c.851G > T/p.(Glu284Val) in Canada	Congenital/Infantile **Late infantile** Juvenile Late juvenile/Protracted Adult *SCAR7*	0–18 mo **2–4 y** 5–10 y >11 y >20 y >4–11 y	Speech delay, seizures, motor decline
CLN3	CLN3 disease	CLN3; lysosome membrane	78	c.461-280_677 + 382del (1-kb deletion)	1 kb intragenic deletion in many countries; 2.8 kb intragenic deletion in Finland	Infantile **Juvenile** Protracted *Autophagic vacuolar myopathy* *Retinitis pigmentosa* *Non-syndromic retinal disease* *Adult cone-rod dystrophy*	0.4 y **5–10 y** >13->40 y >20 y	Rapidly progressing loss of vision, cognitive decline
CLN4 *DNAJC5*	CLN4 disease	DnaJ homologue subfamily C member 5/CSPα, Cysteine string protein; cytoplasm	3		c.346_348delCTC/ p.(Leu116del); c.344T > G/p.(Leu115Arg)	**Adult (autosomal dominant)**	**>20 y**	Seizures, ataxia, behavioural changes
CLN5	CLN5 disease	CLN5; lysosome	37		c.1175_1176del /p.(Tyr392*); c.225G > A/p.(Trp75*) in Finland	Congenital Infantile **Late infantile** Juvenile Protracted Teenage Adult	1.5 y **2–4 y** 5–10 y 17 y >50 y	Slowing of psychomotor development, visual failure
CLN6	CLN6 disease	CLN6; endoplasmic reticulum membrane	82	c.214G > T/p.(Glu72*)	c.461_463del/p.(Ile154del) in Portugal	**Late infantile** Protracted Teenage Adult Kufs type A *Juvenile cerebellar ataxia* *Progressive myoclonus epilepsy*	**2–4 y** >16 y >20->51 y 7–9 y >15	Speech and motor development delay, seizures
CLN7 *MFSD8*	CLN7 disease	major facilitator superfamily domain 8-containing protein; lysosome membrane	46	c.881C > A/ p.(Thr294Lys)	c.881C > A/p.(Thr294Lys) in Roma Gipsies; c.754+2T>A in Eastern Europe	**Late infantile** Juvenile/late juvenile *Nonsyndromic retinal disease* *Adult macular dystrophy* *Adult cone-rod dystrophy*	**2–4 y** 5–11 y >5 y >29–>65 y >27 y	Speech delay, motor difficulties, seizures

(Continued)

TABLE 1 | Continued

Gene	Disease name(s)	Protein and location	Number of reported mutations	Widespread common mutations	Regional-specific mutations	Genotype-phenotype correlation	Age of onset	Presenting and typical clinical features
CLN8	CLN8 disease	CLN8; endoplasmic reticulum membrane	41		c.70C > G/p.(Arg24Gly) in Finland causing EPMR; c.610C > T/p.(Arg204Cys) and c.789G > C/p.(Trp263Cys) in Turkey	**Late infantile** Juvenile *EPMR/Northern epilepsy*	**2–4 y** 6 y 5–10 y	Language and learning difficulties, motor difficulties, seizures
CLN10 *CTSD*	CLN10 disease	cathepsin D; lysosome	10			**Congenital** Infantile Juvenile, late juvenile Teenage	**0 y** 0.5–1.5 y 5–11 y 14–15 y	Seizures, spasticity, central sleep apnoea
CLN11 *GRN*	CLN11 disease	Progranulin, cleaved into granulins; lysosome	3			**Teenage, Adult** *Frontotemporal lobar dementia (when heterozygous)*	**>20 y**	Rapidly progressive visual failure, seizures
CLN13 *CTSF*	CLN13 disease	cathepsin F; lysosome	11			**Adult Kufs type B**	**>20 y**	Tremor, ataxia, seizures

Phenotype bold = phenotype caused by complete loss of gene function.

Age of onset uses the same age range for each gene: congenital (0–0.5y), infantile (0.5–1.5y), late infantile (2–4 yr), juvenile (5–10 yr), late juvenile (11–12y), teenage (13–19y), adult (20+y).

italics–non-NCL disease phenotype that in some cases may be more typically associated with this gene.

CLN9 is not identified. Variations in further genes have been linked with NCL-like phenotypes, these are not included in this table: CLN12/ATP13A2, mutations usually cause Kufor-Rakeb syndrome; CLN14/KCTD7 in cases with infantile and late infantile onset, all other known mutations cause a progressive myoclonic epilepsy or opsoclonus-myoclonus ataxia-like syndrome; SGSH in a case with adult onset, all other known mutations cause MPSIIIA; CLCN6, perhaps modifying disease phenotype (1).

SCAR7, Spinocerebellar Ataxia, Autosomal Recessive 7; EPMR, Epilepsy, Progressive, With Mental Retardation.

CLN6, *CLN7,* and *CLN8* cause disease with onset in late infancy like *CLN2*, the first gene identified causing onset at that age (**Table 1**). *CLN4* was not identified until 2011 (3).

A variety of experimental approaches reflecting the available technology were used to identify NCL genes. The first genes were identified using classic and time-consuming genetic linkage approaches requiring large numbers of similarly affected families followed by positional cloning of the genes [*CLN1* (4) and *CLN3* (5)]. A biochemical approach that detected a missing mannose-6-phosphate tagged lysosomal enzyme in a patient facilitated the identification of *CLN2* (6), alongside ongoing genetic linkage studies. Availability of the human genome sequence meant that going forward fewer families were required to provide sufficient power for genetic linkage analysis, facilitating identification of *CLN5* (7), *CLN6* (8), *CLN7* (9), and *CLN8* (10). Some genes were identified by recognition of stretches of homozygosity in consanguineous families that narrowed the interval that contained the candidate gene. A gene first identified in an animal model led to identification of *CLN10* (11, 12) in human disease. Improvements in sequencing technology later allowed fast and massively parallel sequencing of the whole exome in single families, and facilitated identification of the remaining disease genes [*CLN4* (3), *CLN11* (13), *CLN12* (14), *CLN13* (15), *CLN14* (16)]. The few families suspected of carrying the putative *CLN9* gene were later found to carry mutations in previously identified NCL genes (17, 18).

As monogenic disorders, each NCL is in effect a separate disease entity. All identified NCL genes lie on autosomes. Most cause disease though classic recessive inheritance, where deleterious mutations are present in disease gene alleles inherited from asymptomatic parents. However, adult onset CLN4 disease is dominantly inherited in the few families described with this disease (1, 3). There are three published reports of uniparental disomy in the NCLs, one in which a patient has complete isodisomy of chromosome 8, leading to homozygosity of a maternally-inherited deletion in *CLN8* (19), and two patients for CLN1, both with paternal isodisomy of chromosome 1 (19, 20).

The majority of NCL genes encode proteins that reside in the endo/lysosomal pathways (1, 21–23). Most are lysosomal proteins—enzymes and soluble proteins (*CLN1/PPT1*, *CLN2/TPP1*, *CLN5*, *CLN10/CTSD*, *CLN13/CTSF,* *CLN11/GRN*,) or membrane proteins (*CLN3*, *CLN7/MFSD8*, *CLN12/ATP13A2*). Two encode endoplasmic reticulum membrane proteins *(CLN6*, *CLN8*). Other NCL proteins are cytoplasmic (*CLN4/DNAJC5*, *CLN14/KCTD7*) that peripherally associate with cellular membranes. The *in vivo* substrates for the lysosomal enzymes are incompletely defined, and much remains to be discovered around the functions of the membrane proteins. Nevertheless, recognition of the genetic basis of the NCLs enables the development of targeted therapies even though the underlying disease mechanism for each NCL is not yet fully delineated. It is unlikely that further NCL genes will be identified unless they cause disease in countries where little genetic analysis has been undertaken.

GENOTYPE-PHENOTYPE OBSERVATIONS

NCL Classification

A gene-based classification system was codeveloped by international experts in the NCLs (24) that takes into account the full phenotypic consequences that have emerged over the years, and which includes secondary reference to the age of onset. This replaces the former age-based classification in use since the 1960s. It better supports ongoing gene-based therapeutic development.

There is a classic disease phenotype associated with complete loss of function for most NCL genes, with a typical age of onset and disease progression. The age at which first symptoms appear can be used to guide toward which gene(s) may be mutated. For example, clinically similar NCL disease arising from mutations in more than one gene (e.g., what was originally known as variant late infantile onset NCL) can be caused by loss-of-function mutations in *CLN5*, *CLN6*, *CLN7*, or *CLN8*.

Broad Phenotypes

Most NCL genes actually have a wide age of onset and varied disease courses determined by the underlying mutations (**Table 1**). The increasing implementation of next generation sequencing panels and exome sequencing in diagnosis is leading to more diagnoses of patients with atypical NCL and recognition of these broader phenotypes. These arise from mutations thought or known to have "milder" effects on NCL protein function; and these phenotypes can vary quite considerably. For example, classic CLN6 disease begins in early childhood (late infancy) (8, 25), but disease onset can be delayed as late as adulthood, which also has no associated visual failure (26, 27). Conversely, disease that presents in adulthood caused by mutations in CLN3 may have visual failure as its only or main sign, consistent with this being the presenting symptom for classic juvenile CLN3 disease. Mutations in *CLN7* have been identified in cases of non-syndromic eye disease (28).

This broadening of phenotypes means that disease with a certain age of onset may be caused by loss of function of an NCL protein as well as milder mutations in a gene more usually associated with a younger age of onset. For example, disease beginning in the juvenile age range may be classic CLN3 disease or be juvenile CLN1 disease, or juvenile CLN2, CLN5, CLN6, CLN7, or CLN8 disease.

Distinct Mutation-Specific Phenotypes

Some mutations cause distinct and varied disease that differs from the phenotypes arising from other mutations in the same gene. For example, a single recessive missense mutation in *CLN8* [p.(Arg24Gly)] (10) causes the phenotype described as progressive epilepsy with mental retardation (EPMR) or Northern epilepsy that is found predominantly in Finland. This disease is very different to typical NCL as it is an intellectual developmental disorder that presents with seizures in the juvenile age range that cease in adulthood, and life expectancy is into late adulthood. It was the first genetic disease to be recognised for *CLN8*, with mutations that cause a more typical NCL described later. Similarly, a missense mutation in

CLN2/*TPP1* [p.(Val466Gly)] causes a phenotype first described as spinocerebellar ataxia SCAR7. This is a slowly progressing but not life-limiting disease with no ophthalmologic abnormalities or epilepsy, and without typical ceroid/lipofuscin storage (29). A single gain of function missense mutation in CLCN6 has recently been shown to cause very severe disease in children (30) that would not be classed as NCL, although the mouse model lacking the function of the homologous gene causes mild lysosomal storage disease and the CLCN6 gene was considered a candidate gene for mild NCL disease (31).

There is evidence that the most common and very widespread mutation in *CLN3*, a 1-kb deletion found worldwide accounting for ∼ 90 percent of the affected alleles in CLN3 disease patients (32) does not completely abolish CLN3 function, indeed it may case a gain of function and therefore disease (33, 34). Due to this deletion dominating reports of CLN3 disease, this led to the suspicion that disease caused by complete loss of CLN3 function may not have been described in humans (33). Other distinct phenotypes have been associated with *CLN3* mutations—these include retinitis pigmentosa without other clinical symptoms, even in mid-late adulthood (35) and a distinct disease described as autophagic myopathy associated with heart failure (36). As predicted (33) the phenotype of *CLN3*-associated disease maybe considerably broader (1). There are reports of other families with mutations in some NCL genes that also have predominantly visual problems (28).

Overlap With Other Syndromes

Some mutations in NCL genes cause disease that overlaps with other recognised disease syndromes. This has been described for other rare diseases and more common neurological disorders, such as Niemann-Pick C disease with Alzheimer's disease (37), and type 1 Gaucher disease with Parkinson's disease (38).

Mutations in GRN cause diseases with different types of inheritance. A homozygous recessive (bi-allelic) mutation associated with rectilinear profiles, leads to CLN11 disease, whereas mutations present on one chromosome only cause frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP) (13), which is the second most common type of early-onset dementia. The age of onset and neuropathology of FTLD-TDP and NCL are markedly different, yet there are some shared characteristics: there is autofluorescent, NCL-like storage material in the retina, postmortem brain and lymphoblasts of FTLD-TDP patients (39) and in induced pluripotent stem cells from FTLD-TDP patients (40). Progranulin-deficient mice (13) have features of both NCL and FTLP-TDP diseases (41–43). Therefore, autosomal dominant *GRN* mutations in FTLD-TDP patients cause disease through haploinsufficiency, and it is likely that there are shared disease mechanisms underlying disease in adult CLN11 and FTLD-TDP patients.

Some genes identified as causing NCL more commonly cause inherited diseases given different diagnoses. Mutations in *CLN14*/*KCTD7* cause three different diseases (16, 44–46) classed as progressive myoclonic epilepsy (PME) (47, 48), and in rarer cases PME accompanied by vision loss and lysosomal storage and termed an NCL (16, 49). Mutations in *ATP13A2* typically cause Kufor-Rakeb syndrome and also a late-onset autosomal recessive spastic paraplegia 78 (SPG78) and juvenile onset amyotrophic lateral sclerosis (ALS) (50–52), whereas one family was diagnosed with CLN12 disease (14, 53, 54). Fibroblasts from some SPG78 patients have lysosomal pathology (50). *Atp13a2* knockout mice are reported to accumulate both NCL-type storage material and α-synuclein, and late-onset impairment in sensorimotor functioning. ATP13A2-related disease may therefore represent a disorder with features overlapping both NCL and Parkinson's disease (55). Mutations in *SGSH* usually underlie late infantile onset disease mucopolysaccharidosis type IIIA (MPSIIIA) (56), whereas a mutation in *SGSH* was described in a single case diagnosed with adult onset NCL. Thus, distinctions between inherited disease phenotypes may not be as clear cut as originally anticipated.

There are examples of disease including features of NCL. For example, *CLCN7* underlies a severe autosomal recessive disease combining osteopetrosis, neurodegeneration and lysosomal storage disease (57–59).

Autosomal Dominant Inherited NCL

The clear recessive nature of most NCL had always suggested that mutation carriers are healthy. Given that disease arises in those who are carriers or carrying compound heterozygous mutations in CLN11/GRN, it may be that carriers of mutations in other NCL genes also have deficits. If so, these are likely to be extremely mild or be very late onset and overlap with common features or ageing, and so have not been linked, even anecdotally.

CLN4 disease (Parry disease) is considered autosomal dominant, with disease manifesting in those carrying one of the three mutations in *CLN4* so far described. Disease in humans caused by complete loss of CLN4 function is not known, although the severity of phenotype in animal models with no CLN4 function (60) would predict those carrying biallelic loss-of-function mutations would have very severe and early onset disease. Disease arising from mutations in CLN4/DNAJC5 may therefore be inherited recessively or dominantly.

Multi-Gene Disease

There are a few reports of patients carrying changes in more than one NCL gene. One that was later found to be compound heterozygous for mutations in *CLN5* also carries a single mutation in the *CLCN6* gene that causes recessive NCL in animals (31). Another family is reported in which a single mutation in *CLCN6* is the only described variation; a second heterozygous mutation may be present but not identified. In these two families the *CLCN6* carrier parents were healthy. Some patients carry mutations in more than one gene that underlie variant late infantile NCL (47) (i.e., the mutation database lists changes in CLN5 that have been found alongside those in CLN6 or CLN7 or CLN8). These may be examples of a mutation or specific allele of one gene enhancing or ameliorating the NCL disease phenotype. In mouse NCL models, deletion of both cathepsin B and cathepsin L causes disease, but deletion of either gene alone does not (61).

A patient with disease that presented shortly after birth was found to carry heterozygous mutations in CLN5, together with a mutation in POLG1 that acts to maintain mitochondrial

DNA integrity (62). Increased expression of CLN8 may act as a modifier of Gaucher disease (63). There may be connexions between the function of NCL genes; for example, GRN interacts with CTSD (40), CLN3 affects trafficking of enzymes to the lysosome (64); CLN5 interacts with CLN2 and CLN3 (65).

Human cancer cells acquire somatic mutations in the NCL genes which may confer a growth advantage (*CLN1/PPT1, CLN2/TPP1, CLN3, CLN4/DNAJC5, CLN5, CLN6, CLN7/MFSD8, CLN8, CLN10/CTSD, CLN11/GRN, CLN12/ATP13A2, CLN13/CTSF, CLN14/*KCTD7, as well as *SGSH,* and *CLCN6*) (www.sanger.ac.uk/genetics/CGP/cosmic/) (66). As more sequence variations are deposited through large-scale genome sequencing projects) (e.g., Exome Aggregation Consortium (ExAC): exac.broadinstitute.org/), further correlations may be revealed.

INCIDENCE AND PREVALENCE

NCL are considered the most common inherited neurodegenerative disorder of childhood. They occur worldwide, with some forms first recognised in certain geographical regions. Some types are enriched in or absent from certain regions due to historical population (genetic) bottlenecks.

Incidence and prevalence rates are not available worldwide. Incidence rates are probably more robust than estimated prevalence rates, and generally reported between 1 in 14,000 (Iceland) up to 1 in 100,000 (67). The most common NCL in Northern Europe and the UK are juvenile CLN3 disease and late infantile CLN2 disease, but all types are present.

DIAGNOSTIC IMPLICATIONS

Laboratory Diagnosis

There is an urgency in making an NCL diagnosis now that disease modifying treatments are available or in the pipeline. Biomarkers that follow disease progression and allow the effectiveness of therapies to be monitored are likely to emerge in the near future (68, 69).

New comprehensive approaches are changing the order of diagnostic tests and removing the need for former investigations. Protocols for enzymatic and genetic testing are widely available, making rapid genetic and biochemical diagnosis of most forms of NCL increasingly straightforward (**Table 2**).

Enzyme testing can rapidly confirm deficiencies of CTSD, PPT1, and TPP1 using saliva, blood samples and dried blood spots (70). These enzyme assays should always be applied in cases with an unusual presentation or later onset, and all diagnoses should be supported by DNA sequencing and mutation analysis where possible. For classic juvenile CLN3 disease, the vacuolated lymphocytes which are a common feature, can be visualised by blood film examination (71).

New DNA technologies now allow testing for many genes in a single step regardless of the presentation (70). NCL genes are part of panels designed to interrogate genes underlying a larger group of syndromic and non-syndromic inherited epilepsies. Some common mutations may be screened by DNA-based testing. This can speed earlier diagnosis of NCL before the appearance of other symptoms and also provides a genetic diagnosis for clinically milder or variant phenotypes. As DNA sequencing leads to the description of multiple genetic variation, the genetic cause of atypical disease for some cases will become clearer. Some patients that previously may have been given a diagnosis of NCL may be demonstrated to have atypical forms of other diseases, and *vice versa*. Carrier detection is not possible by histology and is unreliable by enzyme assay; it should always be based on mutation analysis.

Ultrastructural examination of a skin biopsy or blood sample may be helpful for confirmation of NCL disease for atypical forms that are not enzyme deficiencies or do not receive a genetic diagnosis (**Table 1**). Extracerebral storage is readily detected in childhood NCLs but not necessarily in NCL presenting in adulthood (27).

Prenatal Diagnosis

Prenatal diagnosis can be offered to families with a prior history of NCL disease. Preimplantation genetic diagnosis (72) or a combination of enzyme assay and mutational analysis, perhaps with ultrastructural examination of chorionic villus samples obtained at 12–15 weeks gestation, can provide a rapid diagnosis (70).

NCL IN OTHER SPECIES

Some NCL genes are conserved in unicellular or simple organisms, indicating their fundamental function within eukaryotic cells (73). For example, yeasts contain homologous genes to *CLN1/PPT1*, *CLN3 CLN10/CTSD*, *CLN12/ATP13A2.* The slime mould *Dictyostelium discoideum* particularly expresses further NCL gene homologues or members of gene families (e.g., *CLN2/TPP1, CLN4/DNAJC5, CLN5, CLN6, CLN7/MFSD8* family). NCL also occurs in animals (e.g., dogs, sheep, cows, monkey). Cell and animal models carrying mutations in genes equivalent to those causing human NCL are well used in research. These range from yeasts, up to rodents and other mammals (for clinical development). Some of these models are naturally occurring (e.g., mouse, dog, sheep), others are engineered models (e.g., mouse, pig). Some animal NCL disease is caused by mutations in genes not reported to cause similar disease in humans [*ARSG* in dogs (74), *CLCN6* engineered in mice (31), *CTSB*/*CTSL* engineered in mice as double gene mutations (61)].

NCL MUTATION DATABASE

The NCL Mutation Database (www.ucl.ac.uk/ncl-disease) lists known disease-causing mutations and sequence variations in NCL genes by gene and by individual. Five hundred and thirty-seventh NCL disease-causing mutations are currently listed (**Table 2**) across >1,625 patients and >2,160 families. Where possible the age of onset, ethnic background and current location, are listed for each family. Data are gathered from case reports or larger collections in clinical or scientific publications, or referred directly, and updated periodically. These vary in detail according to the report source, e.g., case reports usually have more specifics than reports of large group genetic screens. Mutations are mostly

TABLE 2 | Summary of NCL gene mutations, patients and families currently contained within the NCL Mutation Database.

Gene	CLN1	CLN2	CLN3	CLN4	CLN5	CLN6	CLN7	CLN8	CLN10	CLN11	CLN12	CLN13	CLN14	Grand total
Mutations	71	155	78	3	37	82	46	41	10	3	1	11	1 (13)	537
No. of patients	230	460	442	12	103	145	109	87	18	4	4	15	3 (21)	1,625
No. of families	177	358	418	7	84	118	93	77	13	3	1	9	2 (15)	2,162

CLN12/ATP13A2: mutations usually cause Kufor-Rakeb syndrome, only those causing NCL are shown here; CLN14/KCTD7: mutations cause NCL with infantile and late infantile onset, all other known mutations cause a progressive myoclonic epilepsy or opsoclonus-myoclonus ataxia-like syndrome. Numbers in brackets are mutations that cause a non-NCL phenotype; they are not included in the overall totals.

described in single individuals or occasionally siblings from the same family. Some mutations are more common in certain populations due to local founder effects. Several NCL genes have widespread distribution across several continents due to ancient founder effects (**Table 1**).

An estimate of the proportion of cases caused by each mutation can be made, although there is a considerable under-representation of the occurrence of common mutations since the emphasis is on the collation of novel and rare mutations. The most prevalent mutations are the 1 kb deletion in *CLN3* and two mutations in *CLN2* (1).

Correlations can be drawn between genotype, phenotype and morphological changes in patients, and have been reviewed previously (47, 75), for example for CLN2 disease (76). These derived correlations can be used to predict the disease course in a newly diagnosed family.

This database is important (1). The severity of mutations has implications for treatment. It may be important to know if residual protein or function remains. Treatments may be developed that do not fully compensate for complete loss of gene function and can reduce but not completely eliminate the disease burden—these may be sufficient to improve health in families carrying so-called mild mutations but not in individuals lacking all gene function (2). The location of mutations in the protein may highlight key residues and functional or regulatory domains, aiding understanding of protein function (3). The data reveals the relative frequency of mutations; as ultra-rare, found only within certain ethnic groups, or widespread (4). The data is freely available and contained in excel tables that can be downloaded and used by researchers. For example, there is increasing information on frequency of mutations or disease in specific ethnic groups (4). Efficacy of a new treatment may be demonstrated earlier or more robustly if the mutations and their effects on disease progression of the participants are understood. Going forward, functional data for each mutation can begin to be incorporated, as available.

Other databases exist through international cooperation, enabling collection of natural history data for all NCL types and genotype-phenotype data through databases DEM-CHILD (*www.dem-child.eu*) (77, 78). There are disease rating scales (79–81) to follow disease progression. This is increasing understanding of the genetic spectrum of NCL disease as well as provide necessary control data for use in future clinical trials (77).

CONCLUSION

Most genes that cause NCL disease in humans are probably identified. This, combined with the broader range of associated phenotypes now described, has shown that the genetic picture is considerably more complex than was first envisioned at the start of the genetic era of the NCL. The functions of all NCL genes and thereby disease mechanisms are not yet known. As understanding increases overlap with other rare and common diseases, such as retinal dystrophies may indicate shared disease mechanisms (82).

The gene dosage or the specific mutations show correlation with clinical phenotype. Some variation in clinical phenotype is therefore explained by differing levels of residual protein function. However, variation between families and even siblings shows that co-inheritance of other genetic variations could influence disease phenotype. It is still unclear whether the underlying pathogenic mechanisms are partly shared between classic NCL forms and the alternative disease forms.

The era of genomic medicine is approaching, where genomic information will be used to design the best clinical care for an individual. For the NCL, personalised treatment approaches will be tailored to the underlying mutation and the genetic background of each patient. An early example is the design and delivery of an oligonucleotide therapy for a child with CLN7 disease (83).

Therapeutic development beyond current palliative treatments is advancing slowly. This relies on continued collection of natural history data for the broadening NCL spectrum to provide a control cohort to aid design of future clinical trials. The first approved treatment is for children with classic late infantile CLN2 disease which delivers recombinant protein directly into the brain at regular intervals. For the best long-term clinical benefit for any NCL disease, treatment must begin as early as possible, before any symptoms, which requires rapid and earlier diagnosis using genotype. This may be facilitated by advances in DNA-based approaches that allow future newborn screening (84, 85).

AUTHOR CONTRIBUTIONS

SM devised, interpreted the data, and wrote the review. EG collated the data on mutations, genes and phenotype, and contributed to the writing. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Mole SE, Cotman SL. Genetics of the neuronal ceroid lipofuscinoses (Batten disease). *Biochim Biophys Acta*. (2015) 1852:2237–41. doi: 10.1016/j.bbadis.2015.05.011
2. Zeman W, Dyken P. Neuronal ceroid-lipofuscinosis (Batten's disease): relationship to amaurotic familial idiocy? *Pediatrics.* (1969) 44:570–83.
3. Noskova L, Stranecky V, Hartmannova H, Pristoupilova A, Baresova V, Ivanek R, et al. Mutations in DNAJC5, encoding cysteine-string protein alpha, cause autosomal-dominant adult-onset neuronal ceroid lipofuscinosis. *Am J Hum Genet.* (2011) 89:241–52. doi: 10.1016/j.ajhg.2011.07.003
4. Vesa J, Hellsten E, Verkruyse LA, Camp LA, Rapola J, Santavuori P, et al. Mutations in the palmitoyl protein thioesterase gene causing infantile neuronal ceroid lipofuscinosis. *Nature.* (1995) 376:584–7. doi: 10.1038/376584a0
5. Consortium TIBD. Isolation of a novel gene underlying batten disease, CLN3. *Cell.* (1995) 82:949–57. doi: 10.1016/0092-8674(95)90274-0
6. Sleat DE, Donnelly RJ, Lackland H, Liu CG, Sohar I, Pullarkat RK, et al. Association of mutations in a lysosomal protein with classical late-infantile neuronal ceroid lipofuscinosis. *Science.* (1997) 277:1802–5. doi: 10.1126/science.277.5333.1802
7. Savukoski M, Klockars T, Holmberg V, Santavuori P, Lander ES, Peltonen L. CLN5, a novel gene encoding a putative transmembrane protein mutated in Finnish variant late infantile neuronal ceroid lipofuscinosis. *Nat Genet.* (1998) 19:286–8. doi: 10.1038/975
8. Wheeler RB, Sharp JD, Schultz RA, Joslin JM, Williams RE, Mole SE. The gene mutated in variant late-infantile neuronal ceroid lipofuscinosis (CLN6) and in nclf mutant mice encodes a novel predicted transmembrane protein. *Am J Hum Genet.* (2002) 70:537–42. doi: 10.1086/338708
9. Siintola E, Topcu M, Aula N, Lohi H, Minassian BA, Paterson AD, et al. The novel neuronal ceroid lipofuscinosis gene MFSD8 encodes a putative lysosomal transporter. *Am J Hum Genet.* (2007) 81:136–46. doi: 10.1086/518902
10. Ranta S, Zhang Y, Ross B, Lonka L, Takkunen E, Messer A, et al. The neuronal ceroid lipofuscinoses in human EPMR and *mnd* mutant mice are associated with mutations in CLN8. *Nat Genet.* (1999) 23:233–6. doi: 10.1038/13868
11. Siintola E, Partanen S, Strömme P, Haapanen A, Haltia M, Maehlen J, et al. Cathepsin D deficiency underlies congenital human neuronal ceroid lipofuscinosis. *Brain.* (2006) 129:1438–45. doi: 10.1093/brain/awl107
12. Steinfeld R, Reinhardt K, Schreiber K, Hillebrand M, Kraetzner R, Brück W, et al. Cathepsin D deficiency is associated with a human neurodegenerative disorder. *Am J Hum Genet.* (2006) 78:988–98. doi: 10.1086/504159
13. Smith KR, Damiano J, Franceschetti S, Carpenter S, Canafoglia L, Morbin M, et al. Strikingly different clinicopathological phenotypes determined by progranulin-mutation dosage. *Am J Hum Genet*. (2012) 90:1102–7. doi: 10.1016/j.ajhg.2012.04.021
14. Bras J, Verloes A, Schneider SA, Mole SE, Guerreiro RJ. Mutation of the parkinsonism gene ATP13A2 causes neuronal ceroid-lipofuscinosis. *Hum Mol Genet.* (2012) 21:2646–50. doi: 10.1093/hmg/dds089
15. Smith KR, Dahl HH, Canafoglia L, Andermann E, Damiano J, Morbin M, et al. Cathepsin F mutations cause Type B Kufs disease, an adult-onset neuronal ceroid lipofuscinosis. *Hum Mol Genet*. (2013) 22:1417–23. doi: 10.1093/hmg/dds558
16. Staropoli JF, Karaa A, Lim ET, Kirby A, Elbalalesy N, Romansky SG, et al. A homozygous mutation in KCTD7 links neuronal ceroid lipofuscinosis to the ubiquitin-proteasome system. *Am J Hum Genet.* (2012) 91:202–8. doi: 10.1016/j.ajhg.2012.05.023
17. Haddad SE, Khoury M, Daoud M, Kantar R, Harati H, Mousallem T, et al. CLN5 and CLN8 protein association with ceramide synthase: biochemical and proteomic approaches. *Electrophoresis.* (2012) 33:3798–809. doi: 10.1002/elps.201200472
18. Schulz A, Dhar S, Rylova S, Dbaibo G, Alroy J, Hagel C, et al. Impaired cell adhesion and apoptosis in a novel CLN9 Batten disease variant. *Ann Neurol.* (2004) 56:342–50. doi: 10.1002/ana.20187
19. Travaglini L, Aiello C, Alesi V, Loddo S, Novelli A, Tozzi G, et al. Uniparental disomy of chromosome 1 unmasks recessive mutations of PPT1 in a boy with neuronal ceroid lipofuscinosis type 1. *Brain Dev.* (2016) 9:182–3. doi: 10.1016/j.braindev.2016.08.010
20. Niida Y, Yokoi A, Kuroda M, Mitani Y, Nakagawa H, Ozaki M, et al. A Girl with infantile neuronal ceroid lipofuscinosis caused by novel PPT1 mutation and paternal uniparental isodisomy of chromosome 1. *Brain Dev.* (2016) 38:674–7. doi: 10.1016/j.braindev.2016.01.004
21. Huber RJ, Mathavarajah S. Cln5 is secreted and functions as a glycoside hydrolase in Dictyostelium. *Cell Signal.* (2018) 42:236–48. doi: 10.1016/j.cellsig.2017.11.001
22. Cárcel-Trullols J, Kovács AD, Pearce DA. Cell biology of the NCL proteins: what they do and don't do. *Biochim Biophys Acta.* (2015) 1852:2242–55. doi: 10.1016/j.bbadis.2015.04.027
23. Butz ES, Chandrachud U, Mole SE, Cotman SL. Moving towards a new era of genomics in the neuronal ceroid lipofuscinoses. *Biochim Biophys Acta Mol Basis Dis.* (2020) 1866:165571. doi: 10.1016/j.bbadis.2019.165571
24. Williams RE, Mole SE. New nomenclature and classification scheme for the neuronal ceroid lipofuscinoses. *Neurology.* (2012) 79:183–91. doi: 10.1212/WNL.0b013e31825f0547
25. Gao H, Boustany RM, Espinola JA, Cotman SL, Srinidhi L, Antonellis KA, et al. Mutations in a novel CLN6-encoded transmembrane protein cause variant neuronal ceroid lipofuscinosis in man and mouse. *Am J Hum Genet.* (2002) 70:324–35. doi: 10.1086/338190
26. Arsov T, Smith KR, Damiano J, Franceschetti S, Canafoglia L, Bromhead CJ, et al. Kufs disease, the major adult form of neuronal ceroid lipofuscinosis, caused by mutations in CLN6. *Am J Hum Genet.* (2011) 88:566–73. doi: 10.1016/j.ajhg.2011.04.004
27. Berkovic SF, Staropoli JF, Carpenter S, Oliver KL, Kmoch S, Anderson GW, et al. Diagnosis and misdiagnosis of adult neuronal ceroid lipofuscinosis (Kufs disease). *Neurology.* (2016) 87:579–84. doi: 10.1212/WNL.0000000000002943
28. Roosing S, van den Born LI, Sangermano R, Banfi S, Koenekoop RK, Zonneveld-Vrieling MN, et al. Mutations in MFSD8, encoding a lysosomal membrane protein, are associated with nonsyndromic autosomal recessive macular dystrophy. *Ophthalmology.* (2015) 122:170–9. doi: 10.1016/j.ophtha.2014.07.040
29. Sun Y, Almomani R, Breedveld GJ, Santen GW, Aten E, Lefeber DJ, et al. Autosomal recessive spinocerebellar ataxia 7 (SCAR7) is caused by variants in TPP1, the gene involved in classic late-infantile neuronal ceroid lipofuscinosis 2 disease (CLN2 disease). *Hum Mutat.* (2013) 34:706–13. doi: 10.1002/humu.22292

30. Polovitskaya MM, Barbini C, Martinelli D, Harms FL, Cole FS, Calligari P, et al. A recurrent gain-of-function mutation in CLCN6, encoding the ClC-6 Cl(-)/H(+)-Exchanger, causes early-onset neurodegeneration. *Am J Hum Genet*. (2020) 107:1062–77. doi: 10.1016/j.ajhg.2020.11.004
31. Poët M, Kornak U, Schweizer M, Zdebik AA, Scheel O, Hoelter S, et al. Lysosomal storage disease upon disruption of the neuronal chloride transport protein ClC-6. *Proc Natl Acad Sci USA*. (2006) 103:13854–9. doi: 10.1073/pnas.0606137103
32. Mitchison HM, O'Rawe AM, Taschner PE, Sandkuijl LA, Santavuori P, de Vos N, et al. Batten disease gene, CLN3: linkage disequilibrium mapping in the Finnish population, and analysis of European haplotypes. *Am J Hum Genet*. (1995) 56:654–62.
33. Kitzmuller C, Haines RL, Codlin S, Cutler DF, Mole SE. A function retained by the common mutant CLN3 protein is responsible for the late onset of juvenile neuronal ceroid lipofuscinosis. *Hum Mol Genet.* (2008) 17:303–12. doi: 10.1093/hmg/ddm306
34. Minnis CJ, Townsend S, Petschnigg J, Tinelli E, Bahler J, Russell C, et al. Global network analysis in *Schizosaccharomyces pombe* reveals three distinct consequences of the common 1-kb deletion causing juvenile CLN3 disease. *Sci Rep.* (2021) 11:6332. doi: 10.1038/s41598-021-93446-8
35. Wang F, Wang H, Tuan HF, Nguyen DH, Sun V, Keser V, et al. Next generation sequencing-based molecular diagnosis of retinitis pigmentosa: identification of a novel genotype-phenotype correlation and clinical refinements. *Hum Genet.* (2014) 133:331–45. doi: 10.1007/s00439-013-1381-5
36. Cortese A, Tucci A, Piccolo G, Galimberti CA, Fratta P, Marchioni E, et al. Novel CLN3 mutation causing autophagic vacuolar myopathy. *Neurology*. (2014) 82:2072–6. doi: 10.1212/WNL.0000000000000490
37. Erickson RP, Larson-Thome K, Weberg L, Szybinska A, Mossakowska M, Styczynska M, et al. Variation in NPC1, the gene encoding Niemann-Pick C1, a protein involved in intracellular cholesterol transport, is associated with Alzheimer disease and/or aging in the Polish population. *Neurosci Lett*. (2008) 447:153–7. doi: 10.1016/j.neulet.2008.09.046
38. Aharon-Peretz J, Rosenbaum H, Gershoni-Baruch R. Mutations in the glucocerebrosidase gene and Parkinson's disease in Ashkenazi Jews. *N Engl J Med*. (2004) 351:1972–7. doi: 10.1056/NEJMoa033277
39. Ward ME, Chen R, Huang HY, Ludwig C, Telpoukhovskaia M, Taubes A, et al. Individuals with progranulin haploinsufficiency exhibit features of neuronal ceroid lipofuscinosis. *Sci Transl Med*. (2017) 9:eaah5642. doi: 10.1126/scitranslmed.aah5642
40. Valdez C, Wong YC, Schwake M, Bu G, Wszolek ZK, Krainc D. Progranulin-mediated deficiency of cathepsin D results in FTD and NCL-like phenotypes in neurons derived from FTD patients. *Hum Mol Genet.* (2017) 26:4861–72. doi: 10.1093/hmg/ddx364
41. Gotzl JK, Mori K, Damme M, Fellerer K, Tahirovic S, Kleinberger G, et al. Common pathobiochemical hallmarks of progranulin-associated frontotemporal lobar degeneration and neuronal ceroid lipofuscinosis. *Acta Neuropathol.* (2014) 127:845–60. doi: 10.1007/s00401-014-1262-6
42. Ahmed Z, Sheng H, Xu YF, Lin WL, Innes AE, Gass J, et al. Accelerated lipofuscinosis and ubiquitination in granulin knockout mice suggest a role for progranulin in successful aging. *Am J Pathol.* (2010) 177:311–24. doi: 10.2353/ajpath.2010.090915
43. Yin F, Dumont M, Banerjee R, Ma Y, Li H, Lin MT, et al. Behavioral deficits and progressive neuropathology in progranulin-deficient mice: a mouse model of frontotemporal dementia. *FASEB J.* (2010) 24:4639–47. doi: 10.1096/fj.10.161471
44. Blumkin L, Kivity S, Lev D, Cohen S, Shomrat R, Lerman-Sagie T, et al. A compound heterozygous missense mutation and a large deletion in the KCTD7 gene presenting as an opsoclonus-myoclonus ataxia-like syndrome. *J Neurol.* (2012) 259:2590–8. doi: 10.1007/s00415-012-6545-z
45. Van Bogaert P, Azizieh R, Desir J, Aeby A, De Meirleir L, Laes JF. et al. Mutation of a potassium channel-related gene in progressive myoclonic epilepsy. *Ann Neurol.* (2007) 61:579–86. doi: 10.1002/ana.21121
46. Kousi M, Anttila V, Schulz A, Calafato S, Jakkula E, Riesch E. et al. Novel mutations consolidate KCTD7 as a progressive myoclonus epilepsy gene. *J Med Genet.* (2012) 49:391–9. doi: 10.1136/jmedgenet-2012-100859
47. Kousi M, Lehesjoki AE, Mole SE. Update of the mutation spectrum and clinical correlations of over 360 mutations in eight genes that underlie the neuronal ceroid lipofuscinoses. *Hum Mutat.* (2012) 33:42–63. doi: 10.1002/humu.21624
48. Metz KA, Teng X, Coppens I, Lamb HM, Wagner BE, Rosenfeld JA, et al. KCTD7 deficiency defines a distinct neurodegenerative disorder with a conserved autophagy-lysosome defect. *Ann Neurol.* (2018) 84:766–80. doi: 10.1002/ana.25351
49. Mastrangelo M, Sartori S, Simonati A, Brinciotti M, Moro F, Nosadini M, et al. Progressive myoclonus epilepsy and ceroidolipofuscinosis 14: the multifaceted phenotypic spectrum of KCTD7-related disorders. *Eur J Med Genet.* (2019) 62:103591. doi: 10.1016/j.ejmg.2018.11.025
50. Estrada-Cuzcano A, Martin S, Chamova T, Synofzik M, Timmann D, Holemans T, et al. Loss-of-function mutations in the ATP13A2/PARK9 gene cause complicated hereditary spastic paraplegia (SPG78). *Brain.* (2017) 140:287–305. doi: 10.1093/brain/aww307
51. Spataro R, Kousi M, Farhan SMK, Willer JR, Ross JP, Dion PA, et al. Mutations in ATP13A2 (PARK9) are associated with an amyotrophic lateral sclerosis-like phenotype, implicating this locus in further phenotypic expansion. *Hum Genomics.* (2019) 13:19. doi: 10.1186/s40246-019-0203-9
52. Ramirez A, Heimbach A, Grundemann J, Stiller B, Hampshire D, Cid LP. et al. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. *Nat Genet.* (2006) 38:1184–91. doi: 10.1038/ng1884
53. Tome FM, Brunet P, Fardeau M, Hentati F, Reix J. Familial disorder of the central and peripheral nervous systems with particular cytoplasmic lamellated inclusions in peripheral nerves, muscle satellite cells, and blood capillaries. *Acta Neuropathol.* (1985) 68:209–17. doi: 10.1007/BF00690197
54. De Volder AG, Cirelli S, de Barsy T, Brucher JM, Bol A, Michel C, et al. Neuronal ceroid-lipofuscinosis: preferential metabolic alterations in thalamus and posterior association cortex demonstrated by PET. *J Neurol Neurosurg Psychiatry*. (1990) 53:1063–7. doi: 10.1136/jnnp.53.12.1063
55. Schultheis PJ, Fleming SM, Clippinger AK, Lewis J, Tsunemi T, Giasson B, et al. Atp13a2-deficient mice exhibit neuronal ceroid lipofuscinosis, limited alpha-synuclein accumulation and age-dependent sensorimotor deficits. *Hum Mol Genet*. (2013) 22:2067–82. doi: 10.1093/hmg/ddt057
56. Sleat DE, Ding L, Wang S, Zhao C, Wang Y, Xin W, et al. Mass spectrometry-based protein profiling to determine the cause of lysosomal storage diseases of unknown etiology. *Mol Cell Proteomics.* (2009) 8:1708–18. doi: 10.1074/mcp.M900122-MCP200
57. Kornak U, Kasper D, Bosl MR, Kaiser E, Schweizer M, Schulz A, et al. Loss of the ClC-7 chloride channel leads to osteopetrosis in mice and man. *Cell.* (2001) 104:205–15. doi: 10.1016/S0092-8674(01)00206-9
58. Kasper D, Planells-Cases R, Fuhrmann JC, Scheel O, Zeitz O, Ruether K, et al. Loss of the chloride channel ClC-7 leads to lysosomal storage disease and neurodegeneration. *EMBO J.* (2005) 24:1079–91. doi: 10.1038/sj.emboj.7600576
59. Di Zanni E, Palagano E, Lagostena L, Strina D, Rehman A, Abinun M, et al. Pathobiologic mechanisms of neurodegeneration in osteopetrosis derived from structural and functional analysis of 14 ClC-7 mutants. *J Bone Miner Res.* (2021) 36:531–45. doi: 10.1002/jbmr.4200
60. Fernandez-Chacon R, Wolfel M, Nishimune H, Tabares L, Schmitz F, Castellano-Munoz M, et al. The synaptic vesicle protein CSP alpha prevents presynaptic degeneration. *Neuron.* (2004) 42:237–51. doi: 10.1016/S0896-6273(04)00190-4
61. Stahl S, Reinders Y, Asan E, Mothes W, Conzelmann E, Sickmann A, et al. Proteomic analysis of cathepsin B- and L-deficient mouse brain lysosomes. *Biochim Biophys Acta.* (2007) 1774:1237–46. doi: 10.1016/j.bbapap.2007.07.004
62. Staropoli JF, Xin W, Barone R, Cotman SL, Sims KB. An atypical case of neuronal ceroid lipofuscinosis with co-inheritance of a variably penetrant POLG1 mutation. *BMC Med Genet.* (2012) 13:50. doi: 10.1186/1471-2350-13-50
63. Zhang CK, Stein PB, Liu J, Wang Z, Yang R, Cho JH, et al. Genome-wide association study of N370S homozygous Gaucher disease reveals the candidacy of CLN8 gene as a genetic modifier contributing to extreme phenotypic variation [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Am J Hematol.* (2012) 87:377–83. doi: 10.1002/ajh.23118

64. Metcalfe DJ, Calvi AA, Seamann MNJ, Mitchison HM, Cutler DF. Loss of the Batten disease gene CLN3 prevents exit from the TGN of the mannose 6-phosphate receptor. *Traffic*. (2008) 11:1905–14. doi: 10.1111/j.1600-0854.2008.00807.x
65. Vesa J, Chin MH, Oelgeschlager K, Isosomppi J, DellAngelica EC, Jalanko A, et al. Neuronal ceroid lipofuscinoses are connected at molecular level: interaction of CLN5 protein with CLN2 and CLN3. *Mol Biol Cell*. (2002) 13:2410–20. doi: 10.1091/mbc.e02-01-0031
66. Forbes SA, Bindal N, Bamford S, Cole C, Kok CY, Beare D, et al. COSMIC: mining complete cancer genomes in the catalogue of somatic mutations in cancer [Research Support, Non-U.S. Gov't]. *Nucl Acids Res*. (2011) 39:D945–50. doi: 10.1093/nar/gkq929
67. Mole SE, Williams RE, Goebel HH, (editors.). *The Neuronal Ceroid Lipofusinoses (Batten disease)*. 2nd ed. Oxford: Oxford University Press (2011). p. 444.
68. Kline RA, Wishart TM, Mills K, Heywood WE. Applying modern omic technologies to the neuronal ceroid lipofuscinoses. *Biochim Biophys Acta Mol Basis Dis*. (2020) 1866:165498. doi: 10.1016/j.bbadis.2019.06.012
69. Iwan K, Clayton R, Mills P, Csanyi B, Gissen P, Mole SE, et al. Urine proteomics analysis of patients with neuronal ceroid lipofuscinoses. *iScience*. (2021) 24:102020. doi: 10.1016/j.isci.2020.102020
70. Mole SE, Anderson G, Band HA, Berkovic SF, Cooper JD, Kleine Holthaus SM, et al. Clinical challenges and future therapeutic approaches for neuronal ceroid lipofuscinosis. *Lancet Neurol*. (2019) 18:107–16. doi: 10.1016/S1474-4422(18)30368-5
71. Anderson G, Smith VV, Malone M, Sebire NJ. Blood film examination for vacuolated lymphocytes in the diagnosis of metabolic disorders; retrospective experience of more than 2,500 cases from a single centre. *J Clin Pathol*. (2005) 58:1305–10. doi: 10.1136/jcp.2005.027045
72. Shen J, Cram DS, Wu W, Cai L, Yang X, Sun X, et al. Successful PGD for late infantile neuronal ceroid lipofuscinosis achieved by combined chromosome and TPP1 gene analysis. *Reprod Biomed Online*. (2013) 27:176–83. doi: 10.1016/j.rbmo.2013.04.011
73. Minnis CJ, Thornton CD, FitzPatrick LM, McKay TR. Cellular models of Batten disease. *Biochim Biophys Acta Mol Basis Dis*. (2020) 1866:165559. doi: 10.1016/j.bbadis.2019.165559
74. Abitbol M, Thibaud JL, Olby NJ, Hitte C, Puech JP, Maurer M, et al. A canine Arylsulfatase G (ARSG) mutation leading to a sulfatase deficiency is associated with neuronal ceroid lipofuscinosis. *Proc Natl Acad Sci USA*. (2010) 107:14775–80. doi: 10.1073/pnas.0914206107
75. Mole SE, Williams RE, Goebel HH. Correlations between genotype, ultrastructural morphology and clinical phenotype in the neuronal ceroid lipofuscinoses. *Neurogenet*. (2005) 6:107–26. doi: 10.1007/s10048-005-0218-3
76. Gardner E, Bailey M, Schulz A, Aristorena M, Miller N, Mole SE. Mutation update: review of TPP1 gene variants associated with neuronal ceroid lipofuscinosis CLN2 disease. *Hum Mutat*. (2019) 40:1924–38. doi: 10.1002/humu.23860
77. Schulz A, Ajayi T, Specchio N, de Los Reyes E, Gissen P, Ballon D, et al. Study of intraventricular cerliponase alfa for CLN2 disease. *N Engl J Med*. (2018) 378:1898–907. doi: 10.1056/NEJMoa1712649
78. Nickel M, Simonati A, Jacoby D, Lezius S, Kilian D, Van de Graaf B, et al. Disease characteristics and progression in patients with late-infantile neuronal ceroid lipofuscinosis type 2 (CLN2) disease: an observational cohort study. *Lancet Child Adolesc Health*. (2018) 2:582–90. doi: 10.1016/S2352-4642(18)30179-2
79. Steinfeld R, Heim P, von Gregory H, Meyer K, Ullrich K, Goebel HH, et al. Late infantile neuronal ceroid lipofuscinosis: quantitative description of the clinical course in patients with CLN2 mutations. *Am J Med Genet*. (2002) 112:347–54. doi: 10.1002/ajmg.10660
80. Kwon JM, Adams H, Rothberg PG, Augustine EF, Marshall FJ, Deblieck EA, et al. Quantifying physical decline in juvenile neuronal ceroid lipofuscinosis (Batten disease). *Neurology*. (2011) 77:1801–7. doi: 10.1212/WNL.0b013e318237f649
81. Marshall FJ, de Blieck EA, Mink JW, Dure L, Adams H, Messing S, et al. A clinical rating scale for Batten disease: reliable and relevant for clinical trials. *Neurology*. (2005) 65:275–9. doi: 10.1212/01.wnl.0000169019.41332.8a
82. Cotman SL, Mole SE, Kohan R. Future perspectives: moving towards NCL treatments. *Biochim Biophys Acta*. (2015) 1852:2336–8. doi: 10.1016/j.bbadis.2015.04.001
83. Kim J, Hu C, Moufawad El Achkar C, Black LE, Douville J, Larson A, et al. Patient-customized oligonucleotide therapy for a rare genetic disease. *N Engl J Med*. (2019) 381:1644–52. doi: 10.1056/NEJMoa1813279
84. Barcenas M, Xue C, Marushchak-Vlaskin T, Scott CR, Gelb MH, Turecek F. Tandem mass spectrometry assays of palmitoyl protein thioesterase 1 and tripeptidyl peptidase activity in dried blood spots for the detection of neuronal ceroid lipofuscinoses in newborns. *Anal Chem*. (2014) 86:7962–8. doi: 10.1021/ac501994b
85. Khaledi H, Liu Y, Masi S, Gelb MH. Detection of infantile batten disease by tandem mass spectrometry assay of PPT1 enzyme activity in dried blood spots. *Anal Chem*. (2018) 90:12168–71. doi: 10.1021/acs.analchem.8b03188

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Natural History Studies in NCL and Their Expanding Role in Drug Development: Experiences From CLN2 Disease and Relevance for Clinical Trials

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Conducting clinical trials in rare diseases is challenging. In trials that aim to use natural history control cohorts for evaluation of efficacy, lack of data on natural history of disease prolongs development of future therapies significantly. Therefore, collection of valid natural history data in clinical settings is needed to advance drug development. These data need to fulfill requirements on type of collection, quantifiable measures on the course of disease, verification and monitoring as well as compliance to strict data protection and sharing policies. Disease registries can be a source for patient data. Late-infantile CLN2 disease is characterized by rapid psychomotor decline and epilepsy. Natural-history data of 140 genotype-confirmed CLN2 patients from two independent, international cohorts were analyzed in a natural history study. Both datasets included quantitative ratings with disease-specific clinical scores. Among 41 patients for whom longitudinal assessments spanning an extended disease course were available within the DEM-CHILD DB (an international NCL disease patient database, NCT04613089), a rapid loss of motor and language abilities was documented in quantitative detail. Data showed that the course of disease in late-infantile CLN2 disease is highly predictable with regard to the loss of language and motor function and that the results were homogeneous across multiple and international sites. These data were accepted by EMA and FDA as valid natural-history controls for the evaluation of efficacy in experimental therapies for CLN2 disease and led to an expedited approval of intracerebroventricular enzyme replacement therapy with cerliponase alpha in May 2017.

Keywords: CLN2 disease, NCL, batten disease, natural history studies, rare disease (RD), drug development, neuronal ceroid lipofuscinosis, childhood dementia

INTRODUCTION

Degenerative brain diseases of childhood are a group of rare, heterogeneous diseases including neuronal ceroid lipofuscinoses (NCLs), some forms of mucopolysaccharidoses, leukodystrophies and other rare diseases of inborn errors of metabolism. The majority are relentlessly progressive, incurable and lead to early death. Because they involve loss of developmental skills and intellectual disability, many of them meet the criteria of "childhood dementia" (1, 2). Collection of valid natural history data in these diseases is challenging due to small patient numbers, phenotype variability, missing objective clinical outcome measures and limited disease specific clinical rating scales. Lack of these data prolongs development of future therapies significantly. Natural history of disease data (precisely quantifying progression of disease symptoms and their functional relevance) are therefore needed to advance therapy development.

Overview of NCL Diseases

Neuronal ceroid lipofuscinoses are lysosomal storage disorders characterized by abnormal accumulation of autofluorescent material in lysosomes of the cells (3) and mainly affect retina and gray matter of the cerebral cortex (4). NCL diseases are monogenic diseases and – with one exception – inherited in an autosomal recessive mode. To date, genetic variants in 13 different NCL genes have been described (https://www.ucl.ac.uk/ncl-disease/mutation-and-patient-database). These genes code for lysosomal enzymes, membrane proteins located in different organelles, or other proteins (5). Exactly how deficiencies in these different proteins lead to neurodegeneration and accumulation of lysosomal storage material has not yet been clarified for any of the known NCL diseases. Classification of NCL diseases is currently based on the defective genes and age at first manifestation of symptoms (congenital, infantile, late infantile, juvenile or adult) (5–7). NCLs share common clinical features, in most cases a combination of epilepsy, psychomotor regression with cognitive decline and vision loss. Before onset of symptoms, patients show a largely normal psychomotor development. Most NCL gene defects are assigned a clearly recognizable "classic" or "typical" disease phenotype, which is suggestive of a complete loss of function of the affected protein. Apart from these, a growing number of patients are being identified with "atypical" phenotypes, caused by so-called "mild" genetic variants, that do not lead to a complete loss of function of the corresponding protein.

NCL diseases are diagnosed on the basis of clinical findings, laboratory tests for disease-specific enzymes, cell-morphology studies and genetic testing (4), aiming to identify the pathogenic genetic variants in both alleles of the NCL gene. The increasing use of Next Generation Sequencing Panels and exome sequencing as tools for the diagnosis of rare diseases may lead to the diagnosis of NCL in patients not previously suspected to have the disease. Due to these advancements in genetic testing, the number of atypical, often milder phenotypes described is rapidly increasing especially in NCL forms where a lysosomal enzyme is affected such as CLN2 disease (8–10). Electron-microscopic demonstration of lysosomal storage material in tissues or blood cells can be helpful for detecting an NCL disease. In juvenile CLN3 disease, light microscopy of a blood smear shows characteristic vacuolated lymphocytes. Enzyme testing is a quick and inexpensive way to screen for CLN1 or CLN2 disease. The activity of lysosomal enzymes can be measured in a dry blood spot sample (11). Lack of activity of the enzyme palmitoyl peptidase 1 (PPT1) confirms the diagnosis of CLN1 disease, lack of activity of tripeptidyl peptidase 1 (TPP1) that of CLN2 disease.

Late-Infantile CLN2 Disease

CLN2 disease is caused by genetic variants in the *CLN2* gene that encode for a lysosomal serine protease, tripeptidyl peptidase 1 (TPP1) (12). Loss of TPP1 activity leads to accumulation of ceroid lipofuscin (13, 14). Although there is considerable allelic heterogeneity, two *CLN2* genetic variants are common to more than half of known CLN2 cases in the Western Northern Hemisphere: c.509-1G>A (splicing error) and c.622C>T (non-sense mutation leading to an early stop codon) (5). There is a high genotype-phenotype correlation regarding these variants, leading to a predominantly late-infantile phenotype. Many rarer *CLN2* genetic variants have been reported whose influence on phenotype and residual TPP1 activity is not well understood; some genetic variants are thought to result in the delayed onset or prolonged course of the disease (15). Atypical CLN2 phenotypes are reported more frequently, with increased ability for genetic testing from different regions in the world (8, 10, 15–22). In Latin America 50% of cases show protracted course of disease with later onset of symptoms and slightly different order of symptom onset (9).

Late-infantile CLN2 disease (OMIM# 204500) globally has been the most common phenotype in CLN2 disease. It is characterized by a severe and rapidly progressive neurodegenerative syndrome with onset most common in children aged 2–4 years (23). Epileptic seizures, loss of language, motor function and cognition, blindness and premature death (14, 24) quickly follow the onset of first symptoms. Photoparoxysmal response (PPR) on intermittent photic stimulation at low stimulation frequencies of 1-3 Hz can be an early hallmark (25). CLN2 disease occurs worldwide, but data on incidence and prevalence are scarce (26–28). Because the first symptoms (language delay, epileptic seizures, delay of psychomotor development and ataxia) are non-specific to CLN2 and are sometimes found in children with epilepsy as side effects of anti-seizure medications (ASMs), they are commonly misinterpreted, and diagnosis is often delayed. Vision loss, a characteristic clinical sign for NCL, occurs only in the late stages of late-infantile CLN2 disease and therefore is not a clinical hallmark for early diagnosis. The burden of CLN2 disease is high, not only emotionally for affected families but also for society at large. A missed diagnosis prevents parents from receiving genetic counseling for future planned pregnancies and may lead to their having subsequent children who are also affected.

TABLE 1 | Overview of types of data (static/retrospective, dynamic/prospective).

Static data (retrospective)	Dynamic data (mainly prospective)
• Demographics • Genetic Diagnosis • Medical history (age at diagnosis, onset of symptoms, type of first symptoms) • Age at achievement of milestones of psychomotor development	• Disease specific rating scales (see **Table 2**) • MRI/OCT/EEG/other diagnostic evaluations • Clinical examinations (standardized neurodevelopmental exams, etc.) • QoL Questionnaires

NATURAL HISTORY DATA IN CLINICAL TRIALS FOR RARE DISEASES

Regulatory Agency Recommendations on Natural History Data Used in Clinical Trials

Since the number of rare diseases is rising, with advanced diagnostic methods and genetic testing available, development of new drugs for the unmet medical need of millions of patients is needed. Natural history data can serve as control data in clinical trials and therefore shorten time in drug development. Therefore, regulatory agencies, such as FDA (29) and EMA (30), have released guidelines to ensure high quality of natural history data for drug development in rare diseases.

Key points of these guidelines are:

- DATA SUITABILITY: data collection, data storage, data extraction and quality control processes need to fulfill the basic rules of GCP (good clinical practice) guidelines for clinical trials. Any source data need to be dated and signed and digital records have to be traceable with audit trails. Most importantly, informed consent processes for participation in a scientific data collection and international data sharing need to be in place, considering local ethical requirements as well as privacy issues and governance-related issues.
- PATIENT POPULATION AND BIOMARKERS: genotypic and/or phenotypic heterogeneity can affect the characterization, progression, and physiological changes of the disease. Therefore, such information is important for the development of clinical biomarkers for diagnosis, prognosis of disease progression, and prediction of treatment response. In addition, it might guide patient and dose selection in clinical trials. Disease specific centers of excellence should guide these data.
- CLINICAL OUTCOME ASSESSMENTS: can assess both safety and efficacy and may include observer-reported, patient-reported, caregiver-reported, and performance outcome measures. Natural history studies can combine information from patient medical records and other existing sources of disease-specific information. Longitudinal retrospective and prospective (longitudinal) studies can help fill critical gaps in knowledge of disease progression and set a course for future analysis. In these studies data are collected over time, making them more suitable for use as an external control group. Cross-sectional studies collect patient data at a specific time point offering a snapshot of disease and can be used to support existing data.

EMA guidelines specifically focus on data collected in disease registries, which is defined "as an organized system that collects data and information on a group of people defined by a particular disease or condition, and that serves a pre-determined scientific, clinical and/or public health (policy) purpose". In comparison, FDA guidelines are kept more general in use of natural history study data provided. Both guidelines share similar keypoints though regarding quality and use of data and encourage the use of clinically collected natural history data in rare diseases for use in drug development.

Methods Established in Natural History Data Collection for NCLs

Disease Specific Center of Excellence

For over 20 years, the Hamburg Specialty Center for NCL and related childhood dementias has been established and is treating around 170 patients annually with all types of NCL diseases. The DEM-CHILD database, an NCL disease registry, was founded in Hamburg as part of the EU-funded FP7 project DEM-CHILD ("A Treatment-Oriented Research Project of NCL Disorders as a Major Cause of **Dem**entia in **Child**hood", GAN°281234, www.dem-child.eu). The DEM-CHILD project focused on the main cause for childhood dementia in Europe, the neuronal ceroid lipofuscinoses (NCLs). In order to advance the development of treatment options for NCL diseases, the DEM-CHILD project combined the expertise of (i) recognized European research teams, both basic scientists and clinicians, (ii) high-technology SMEs, (iii) experts in medical ethics, and (iv) NCL patients and family associations. The project implemented a novel network including the most prominent NCL researchers, both basic scientists and clinicians, in Europe, collaborating with Indian experts, to collect the world largest, clinically and genetically best characterized set of NCL patients. One work package was (i) to establish an NCL mutation and NCL patient registry of long-lasting function to describe accurately and in detail the clinical course and clinical spectrum, as well as genotype-phenotype variability in different forms of NCL; and (ii) to establish a tool for the evaluation of experimental therapy studies in the NCLs.

The International DEM-CHILD Database

Only patients with a genetically verified diagnosis of an NCL disease can be included in the data collection after signing an informed consent for participation.

Following the successful application for an international natural-history study for all NCL diseases (www.clinicaltrials.gov, NCT04613089), the DEM-CHILD database is nowadays able to collect data internationally. For rare diseases, such global cooperation is desperately needed for building robust datasets for future therapeutic trials. Different types of data are collected within the DEM-CHILD database, separated into "static" and "dynamic" datasets. Static data do not change over time (e.g., genetic diagnosis, age at first symptoms and timepoints of reached

TABLE 2 | Overview of disease specific rating scales for NCLs.

Clinical rating scale (CRS)	NCL phenotype	Clinical categories	Type of analysis possible		No of patients in original publication	Reference
			Retrospective	Prospective		
Hamburg INCL score	infantile	Mobility Fine motor function Expressive language (add on categories: communication/interaction, sleep, agitation/irritability, seizures, feeding, visual attention)	✓	✓	14	(31)
Hamburg LINCL score	late-infantile	Motor Language Seizures Vision	✓	✓	22	(32)
Hamburg JNCL score	juvenile	Motor Language Epilepsy Vision Intellect	✓	✓	17	(33)
Weill-Cornell (WCMC)	late-infantile	Motor Language Gait Feeding	*X*	✓	18	(34)
CLN2 clinical rating scale ML (CLN2-CRS ML)	late-infantile	Motor Language	*X*	✓	24	(35)
Expanded CLN2 clinical rating scale (CLN2-CRS MX-LX)	late-infantile	Motor Language	*X*	✓	30	(36)
Unified batten disease rating scale (UBDRS)	juvenile	Physical Seizures Behavior Capability Clinical global impression	*X*	✓	31, 82	(37, 38)
Opthalmologic: Weill-Cornell LINCL opthalmic scale (WCBS)	late-infantile	Color/fundus OCT Fluorescein/indocyanine green angiogram (FA/ICGA)	✓*	✓	25	(39)
Opthalmologic: Hamburg CLN3 opthalmic rating scale	juvenile	Visual acuity/BCVA Fundus score OCT	✓*	✓	21	(40)
Pain: battens observational pain scale (BOPS)	not specified	Pain	*X*	✓	35	(41)

**If images retrospectively have been collected for OCT, FA and ICGA.*

milestones in psychomotor development as well as their loss) whereas dynamic data are related to an exam date and might change over time with progression of disease (**Table 1**). Examples for dynamic data are clinical scoring data on disease progression (e.g., disease specific clinical rating scales (**Table 2**) and all examinations conducted at a defined timepoint. The static data sets represent the background for the analysis of the dynamic data. They allow the interpretation of different disease progression rates based on the genetic diagnosis and thereby lead to a better understanding of the genotype-phenotype correlation. In addition, detailed analysis of the retrospective static data has led to the identification of early symptoms supporting early diagnosis.

Clinical Rating Scales in NCL Diseases

The development of new therapies requires an exact understanding of the clinical course of the disease needed to treat, and its variability across a wide range of patients. Limited quantifiable data make statistical analysis and extrapolation difficult. To date, only a few reports have provided a quantitative description of the clinical course of disease in NCLs. Disease specific clinical rating scales have been developed for infantile, late-infantile and juvenile phenotypes. Rating severity and progression of key symptoms of disease, these can be applied prospectively, retrospectively or combined, depending on the specific scale (**Table 2**). In addition, novel ophthalmologic scales also focus on rating progression of vision loss and changes in retinal function and histology as presented in **Table 2** below.

Role of Natural History Data in Drug Development for CLN2 Disease

Until recently, only palliative treatment had been available for CLN2 patients.

Therefore, the aim of a collaborative natural history data collection for CLN2 disease was to create a precise dataset on disease progression which should ultimately serve as control data for experimental therapy studies.

In an international natural history collection of 140 patients with late-infantile CLN2 disease (42), 41 patients from the DEM-CHILD DB from Hamburg could provide a longitudinal quantitative core data set, with clinical ratings throughout the long course of the disease. Patient cohorts from Italy (Verona) and the US (Weill Cornell Medical College) provided predominantly cross-sectional or short-time longitudinal data sets in addition. Acquired in a multi-site international setting, the findings demonstrated that the rate of disease progression was homogeneous for all data sets and that, despite subjects being located in different countries and being independently rated, the results were highly reproducible across the cohorts.

These CLN2 natural history data have successfully been used as natural-history controls in a clinical trial testing safety and efficacy of intraventricular enzyme replacement therapy with cerliponase alfa (43). Comparison of data from treated patients with these natural history data showed that this treatment was able to slow down disease progression. Subsequently, these data were monitored and approved as part of the market approval process of cerliponase alfa by EMA, FDA and PMDA.

DISCUSSION

Valid longitudinal and internationally collected natural history data, that can quantify disease progression in rare diseases, are urgently needed for evaluating the efficacy of current and future therapies and to shorten time to approval of new therapeutic options. Collection of these data face multiple challenges when used as comparative cohorts for efficacy in clinical trials. Nevertheless, these challenges are known and can be addressed in order to internationally pool existing data and to give patients the opportunity to contribute own data to achieve the overall goal of trial readiness in a rare disease. For a majority of parents and patients, the knowledge of helping to advance new therapies for future effected families by providing own data on the course of disease, helps in coping with these devastating progressive diseases like NCL. The motivation of these families is therefore high in order to help for a greater good and should be addressed by making it possible that not only patients in excellent health care systems are able to join via expert centers, but are able to provide needed data by themselves that can be validated centrally by experts in the field.

International data collection collaborations had been slowed across all diseases by the new European Data Protection Regulations (GDPR) that came into effect in May 2018. The development of new guidelines on sharing data had to be implemented, local ethic approvals renewed, and individual patients had to be re-consented for international collaboration on sharing of data. Subsequently this may have helped to streamline the collaborative use of identical tools for evaluating the course of disease and the type of data collected. In using the same tools, these can be developed further and adapted to individual center needs, moreover though, it makes comparison between centers objective and evaluation of data sets uncomplicated.

In order to be able to use data acquired in clinical settings for use in clinical trials, these need to meet the same quality requirements as data collected in clinical trials (monitorable) in order to be auditable and approved by health care authorities. Written reports signed with date and digital records that have audit trails implemented within the systems are standards that need to be in place.

Evaluating rare diseases and quantifying progression of disease nevertheless remains challenging and has its limitations in use. One challenge is the limited sample size in such diseases, another the mostly retrospective nature of longitudinal data with varying timepoints of data collection and its retrieval from medical charts and country dependent obligatory childhood exams.

Nevertheless, these challenges can be overcome. The natural history data collection in late-infantile CLN2 disease by Nickel et al. (42), investigated the largest cohort so far for late-infantile CLN2 disease of quantitative natural-history data, acquired in a multi-site international setting. Before this collaboration, data describing the natural history of late-infantile CLN2 disease were very limited: previous studies either contained mostly retrospectively collected data or were restricted to cross-sectional data only. Moreover, these previous studies analyzed data sets from one center only without providing evidence that the data were representative across multiple study sites and in an international setting. Thus, the data from this natural history study represent an important milestone in quantifying the progression of neurodegeneration in children with late-infantile CLN2 disease according to an editorial comment by Mink in 2018 (44).

Data from this study have successfully served as natural-history control data in completed experimental therapy trials on intraventricular ERT for CLN2 disease (43) and are also being used in ongoing experimental therapy trials on intraventricular ERT (NCT01907087, NCT02485899, NCT02678689) and intraparenchymal adeno-associated gene therapies [(45), NCT00151216, NCT01161576, NCT01414985]. Intraventricular ERT has recently been approved in the US, Europe and Japan as treatment for CLN2 disease [(46–48), online] on the basis of comparisons with these natural-history data. They will also be valuable for any clinical trials looking at future treatment developments for CLN2 disease such as other forms of gene therapy or new pharmacological approaches.

AUTHOR CONTRIBUTIONS

MN and AS contributed to conception and design of the study, writing of the manuscript as well as manuscript revision, read, and approved the submitted version.

REFERENCES

1. Shapiro EG, Klein KA. Dementia in childhood: issues in neuropsychological assessment with application to the natural history and treatment of degenerative storage diseases. In: Tramontana MG, Hooper SR, editors. *Advances in Child Neuropsychology*. 1st edition, New York: Springer (1994). p. 119–71.
2. Kohlschütter A, Schulz A, Nickel M, Richterich A. Demenzerkrankungen bei Kindern und Jugendlichen. *Päd Praxis.* (2015) 83:561–70.
3. Radke A, Stenzel W, Goebel HH. Human NCL neuropathology. *Biochim Biophys Acta*. (2015) 1852:2262–6. doi: 10.1016/j.bbadis.2015.05.007
4. Schulz A, Kohlschütter A, Mink J, Simonati A, Williams R. NCL diseases-clinical perspectives. *Biochim Biophys Acta.* (2013) 1832:1801–6. doi: 10.1016/j.bbadis.2013.04.008
5. Mole SE, Cotman SL. Genetics of the neuronal ceroid lipofuscinoses (Batten disease). *Biochim Biophys Acta*. (2015) 1852:2237–41. doi: 10.1016/j.bbadis.2015.05.011
6. Williams RE, Mole SE. New nomenclature and classification scheme for the neuronal ceroid lipofuscinoses. *Neurology.* (2012) 79:183–91. doi: 10.1212/WNL.0b013e31825f0547
7. Mole SE, Anderson G, Band HA, Berkovic SF, Cooper JD, Kleine Holthaus SM, et al. Clinical challenges and future therapeutic approaches for neuronal ceroid lipofuscinosis. *Lancet Neurol.* (2019) 18:107–16. doi: 10.1016/S1474-4422(18)30368-5
8. Kohan R, Carabelos MN, Xin W, Sims K, Guelbert N, Cismondi IA, et al. Neuronal ceroid lipofuscinosis type CLN2: a new rationale for the construction of phenotypic subgroups based on a survey of 25 cases in South America. *Gene.* (2013) 516:114–21. doi: 10.1016/j.gene.2012.12.058
9. Lourenço CM, Pessoa A, Mendes CC, Rivera-Nieto C, Vergara D, Troncoso M, et al. Revealing the clinical phenotype of atypical neuronal ceroid lipofuscinosis type 2 disease: Insights from the largest cohort in the world. *J Paediatr Child Health.* (2021) 57:519–25. doi: 10.1111/jpc.15250
10. Elleder ML, Dvorakova L, Stolnaja H, Vlaskova H, Hulkova R, Druga H, et al. Atypical CLN2 with later onset and prolonged course: a neuropathologic study showing different sensitivity of neuronal subpopulations to TPP1 deficiency. *Acta Neuropathol*. (2008) 116:119–24. doi: 10.1007/s00401-008-0349-3
11. Lukacs ZM, Nickel S, Murko P, Nieves Cobos A, Schulz A, Santer R, et al. Validity of a rapid and simple fluorometric tripeptidyl peptidase 1 (TPP1) assay using dried blood specimens to diagnose CLN2 disease. *Clin Chim Acta*. (2019) 492:69–71. doi: 10.1016/j.cca.2019.02.010
12. Sleat DE, Donnelly RJ, Lackland H, Liu CG, Sohar I, Pullarkat RK, et al. Association of mutations in a lysosomal protein with classical late-infantile neuronal ceroid lipofuscinosis. *Science.* (1997) 277:1802–5. doi: 10.1126/science.277.5333.1802
13. Haltia M. The neuronal ceroid-lipofuscinoses. *J Neuropathol Exp Neurol.* (2003) 62:1–13. doi: 10.1093/jnen/62.1.1
14. Mole SE, Williams RE, Goebel HH. Correlations between genotype, ultrastructural morphology and clinical phenotype in the neuronal ceroid lipofuscinoses. *Neurogenetics.* (2005) 6:107–26. doi: 10.1007/s10048-005-0218-3
15. Gardner E, Bailey M, Schulz A, Aristorena M, Miller N, Mole SE. Mutation update: Review of TPP1 gene variants associated with neuronal ceroid lipofuscinosis CLN2 disease. *Hum Mutat.* (2019) 40:1924–38. doi: 10.1002/humu.23860
16. Bessa C, Teixeira CA, Dias A, Alves M, Rocha S, Lacerda L, et al. CLN2/TPP1 deficiency: the novel mutation IVS7-10A>G causes intron retention and is associated with a mild disease phenotype. *Mol Genet Metab*. (2008) 93:66–73. doi: 10.1016/j.ymgme.2007.08.124
17. Kousi M, Lehesjoki A-E, Mole SE. Update of the mutation spectrum and clinical correlations of over 360 mutations in eight genes that underlie the neuronal ceroid lipofuscinoses. *Hum Mutat*. (2012) 33:42–63. doi: 10.1002/humu.21624
18. Di Giacopo RL, Cianetti V, Caputo I, La Torraca F, Piemonte A, Ciolfi S, et al. Protracted late infantile ceroid lipofuscinosis due to TPP1 mutations: Clinical, molecular and biochemical characterization in three sibs. *J Neurol Sci.* (2015) 356:65–71. doi: 10.1016/j.jns.2015.05.021
19. Saini AG, Sankhyan N, Singhi P. Chorea in late-infantile neuronal ceroid lipofuscinosis: an atypical presentation. *Pediatr Neurol.* (2016) 60:75–8. doi: 10.1016/j.pediatrneurol.2016.02.015
20. Chen Z-R, Liu D-T, Meng H. Homozygous missense TPP1 mutation associated with mild late infantile neuronal ceroid lipofuscinosis and the genotype-phenotype correlation. *Seizure*. (2019) 69:180–5. doi: 10.1016/j.seizure.2018.08.027
21. Johnson AM, Mandelstam S, Andrews I, Boysen K, Yaplito-Lee J, Fietz M, et al. Neuronal ceroid lipofuscinosis type 2: an Australian case series. *J Paediatr Child Health.* (2020) 56:1210–8. doi: 10.1111/jpc.14890
22. Wibbeler E, Wang R, Reyes EL, Specchio N, Gissen P, Guelbert N, et al. Cerliponase alfa for the treatment of atypical phenotypes of CLN2 disease: a retrospective case series. *J Child Neurol.* (2021) 36:468–74. doi: 10.1177/0883073820977997
23. Chang M, Cooper JD, Davidson BL, van Diggelen OP, Elleder M, Goebel HH, et al. CLN2. In: Mole SE, Williams RE, Goebel HH, editors. *The Neuronal Ceroid Lipofuscinoses (Batten Disease)*, 2nd edition, Oxford: University Press (2011). p. 80–109.
24. Kohlschütter A, Schulz A. CLN2 Disease (Classic Late Infantile Neuronal Ceroid Lipofuscinosis). *Pediatr Endocrinol Rev.* (2016) 13 Suppl 1:682–8.
25. Specchio N, Bellusci M, Pietrafusa N, Trivisano M, de Palma L, Vigevano F. Photosensitivity is an early marker of neuronal ceroid lipofuscinosis type 2 disease. *Epilepsia*. (2020) 58:1380–8. doi: 10.1111/epi.13820
26. Claussen M, Heim P, Knispel J, Goebel HH, Kohlschütter A. Incidence of neuronal ceroid-lipofuscinoses in West Germany: variation of a method for studying autosomal recessive disorders. *Am J Med Genet*. (1992) 42:536–8. doi: 10.1002/ajmg.1320420422
27. Uvebrant P, Hagberg B. Neuronal ceroid lipofuscinoses in Scandinavia. Epidemiology and clinical pictures. *Neuropediatrics.* (1997) 28:6–8. doi: 10.1055/s-2007-973654
28. Moore SJ, Buckley DJ, MacMillan A, Marshall HD, Steele L, Ray PN, et al. The clinical and genetic epidemiology of neuronal ceroid lipofuscinosis in Newfoundland. *Clin Genet.* (2008) 74:213–22. doi: 10.1111/j.1399-0004.2008.01054.x
29. FDA: 01MARCH2019 (online). Available online at: https://www.fda.gov/media/122425/download (accessed september 26, 2021).
30. EMA: 24SEPTEMBER2020 (online). Available online at: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-registry-based-studies_en.pdf (accessed september 26, 2021).
31. Nickel M, Schwering C, Westermann L, Wibbeler E, Lezius S, Schulz A. Hamburg iNCL scale: A new tool for the quantitative description of disease progression in infantile CLN1 patients. In: *Presented at 17th international Congress on Neuronal Ceroid Lipofuscinosis,* Oct 6th-10th 2021, St. Louis (2021).
32. Steinfeld R, Heim P, von Gregory H, Meyer K, Ullrich K, Goebel HH, et al. Late infantile neuronal ceroid lipofuscinosis: quantitative description of the clinical course in patients with CLN2 mutations. *Am J Med Genet.* (2002) 112:347–54. doi: 10.1002/ajmg.10660
33. Kohlschütter A, Laabs R, Albani M. Juvenile neuronal ceroid lipofuscinosis (JNCL): quantitative description of its clinical variability. *Acta Paediatr Scand*. (1988) 77:867–72. doi: 10.1111/j.1651-2227.1988.tb10770.x
34. Worgall S, Kekatpure MV, Heier L, Ballon D, Dyke JP, Shungu D, et al. Neurological deterioration in late infantile neuronal ceroid lipofuscinosis. *Neurology*. (2007) 69:521–35. doi: 10.1212/01.wnl.0000267885.47092.40
35. Wyrwich KW, Schulz A, Nickel M, Slasor P, Ajayi T, Jacoby DR, et al. An Adapted Clinical Measurement Tool for the Key Symptoms of CLN2 Disease. *J Inborn Errors Metab Screen*. (2018) 6:1–7. doi: 10.1177/2326409818788382
36. Phillips D, Cho Y, Nevoret M-L, Wood M, Nickel M, Schwering C, et al. The expanded neuronal ceroid lipofuscinosis 2 (CLN2) clinical rating scale for motor and language function: development and inter-rater reliability. In: *Accepted for Posterpresentation at 17th international Congress on Neuronal Ceroid Lipofuscinosis*, Oct 6th-10th 2021, St. Louis (2021).
37. Marshall FJ, de Blieck EA, Mink JW, Dure L, Adams H, Messing S, et al. A clinical rating scale for Batten disease: reliable and relevant for clinical trials. *Neurology*. (2005) 65:275–9. doi: 10.1212/01.wnl.0000169019.41332.8a

38. Kwon JM, Adams H, Rothberg PG, Augustine EF, Marshall FJ, Deblieck EA, et al. Quantifying physical decline in juvenile neuronal ceroid lipofuscinosis (Batten disease). *Neurology*. (2011) 77:1801–7. doi: 10.1212/WNL.0b013e318237f649
39. Orlin A, Sondhi D, Witmer MT, Wessel MM, Mezey JG, Kaminsky SM, et al. Spectrum of ocular manifestations in CLN2-associated batten (Jansky-Bielschowsky) disease correlate with advancing age and deteriorating neurological function. *PLoS ONE*. (2013) 8:e73128. doi: 10.1371/journal.pone.0073128
40. Dulz S, Atiskova Y, Wibbeler E, Wildner J, Wagenfeld L, Schwering C, et al. An ophthalmic rating scale to assess ocular involvement in juvenile CLN3 disease. *Am J Ophthalmol*. (2020) 220:64–71. doi: 10.1016/j.ajo.2020.07.015
41. Breau L, Camfield C, Camfield P. Development and initial validation of the Batten's Observational Pain Scale: a preliminary study. *J Pain Manag*. (2010) 3:283–92. doi: 10.1037/t68970-000
42. Nickel M, Simonati A, Jacoby D, Lezius S, Kilian D, Van de Graaf B, et al. Disease characteristics and progression in patients with late-infantile neuronal ceroid lipofuscinosis type 2 (CLN2) disease: an observational cohort study. *Lancet Child Adolesc Health*. (2018) 2:582–90. doi: 10.1016/S2352-4642(18)30179-2
43. Schulz A, Ajayi T, Specchio N, de Los Reyes E, Gissen P, Ballon D, et al. Study of intraventricular cerliponase alfa for CLN2 disease. N Engl J Med. (2018) 378:1898–907. doi: 10.1056/NEJMoa1712649
44. Mink JW. Natural history data for childhood neurodegenerative disease. *Lancet Child Adolesc Health*. (2018) 2:547–8. doi: 10.1016/S2352-4642(18)30210-4
45. Sondhi D, Kaminsky SM, Hackett NR, Pagovich OE, Rosenberg JB, De BP, et al. Slowing late infantile Batten disease by direct brain parenchymal administration of a rh10 adeno-associated virus expressing CLN2. *Sci Transl Med*. (2020) 12:eabb5413. doi: 10.1126/scitranslmed.abb5413
46. FDA: 27APRIL2017 (online). Available online at: https://www.fda.gov/news-events/press-announcements/fda-approves-first-treatment-form-batten-disease (accessed January 16, 2021).
47. EMA: 21APRIL2017 (online). Available online at: https://www.ema.europa.eu/en/news/new-medicine-rare-neurodegenerative-disorder-children (accessed January 16, 2021).
48. PMDA: 20SEPTEMBER2019 (online). Available online at: https://www.pmda.go.jp/files/000235289.pdf (accessed January 16, 2021).

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Neurophysiological Findings in Neuronal Ceroid Lipofuscinoses

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Neuronal ceroid lipofuscinoses (NCLs) are a heterogeneous group of neurodegenerative diseases, characterized by progressive cerebral atrophy due to lysosomal storage disorder. Common clinical features include epileptic seizures, progressive cognitive and motor decline, and visual failure, which occur over different time courses according to subtypes. During the latest years, many advances have been done in the field of targeted treatments, and in the next future, gene therapies and enzyme replacement treatments may be available for several NCL variants. Considering that there is rapid disease progression in NCLs, an early diagnosis is crucial, and neurophysiological features might have a key role for this purpose. Across the different subtypes of NCLs, electroencephalogram (EEG) is characterized by a progressive deterioration of cerebral activity with slowing of background activity and disappearance of spindles during sleep. Some types of heterogeneous abnormalities, diffuse or focal, prevalent over temporal and occipital regions, are described in many NCL variants. Photoparoxysmal response to low-frequency intermittent photic stimulation (IPS) is a typical EEG finding, mostly described in CLN2, CLN5, and CLN6 diseases. Visual evoked potentials (VEPs) allow to monitor the visual functions, and the lack of response at electroretinogram (ERG) reflects retinal neurodegeneration. Taken together, EEG, VEPs, and ERG may represent essential tools toward an early diagnosis of NCLs.

Keywords: neuronal ceroid lipofuscinoses, EEG, photoparoxysmal response, intermittent photic stimulation, visual evoked potentials, electroretinogram, neurophysiological findings

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INTRODUCTION

Neuronal ceroid lipofuscinoses (NCLs) are a heterogeneous group of autosomal recessive neurodegenerative disorders, characterized by progressive cerebral atrophy due to widespread accumulation of autofluorescent storage material within lysosomes (1–5). NCLs are caused by defective lysosomal processing enzymes or receptors (3–8). So far, 14 variants of NCLs are reported; they share some common clinical features including epileptic seizures, progressive cognitive and motor decline, and visual failure; all those symptoms occur over different time courses according to subtypes (9, 10).

Neuronal ceroid lipofuscinoses represent the most common cause of dementia in children (5, 8, 11), and the incidence varies worldwide from 1:12,500 to 1:100,000 (9).

Neuronal ceroid lipofuscinoses can be often misdiagnosed at the onset because of the appearance of non-specific presenting symptoms; therefore, the diagnosis may be delayed (5).

Clinical management is mostly palliative, although, in the recent years, many efforts have been done to identify targeted treatments, i.e., enzymatic replacement therapy (ERT) and genetic therapy for each of NCL variants (7, 12–16). Nevertheless, a targeted treatment with human recombinant enzyme is currently available only for CLN2 disease (12).

Considering the rapid disease progression (17) and the development of novel targeted treatments, early diagnosis remains crucial. Early diagnosis is also useful for genetic counseling to avoid family odyssey toward different hospitals looking for diagnosis and to avoid repetition of useless investigations (18). Next-generation sequencing techniques are successful in the identification of genetic childhood epilepsies and early detection of CLN2 disease (19). A recent study supported the value of target re-sequencing in patients with genetic childhood epilepsies, suggesting that this technique may be successful in the early detection of patients with CLN2 (19).

In this context, neurophysiological data may have a key role in the diagnosis of NCLs. Electroencephalogram (EEG) and evoked potentials may be of particular importance for this purpose, since they can provide critical information at a relatively early phase of the disease and are easy to carry out in a short time and without excessive costs.

This review aims to provide neurophysiological findings in NCLs, highlighting the neurophysiologic typical features of NCLs as well as what may allow differential diagnosis with developmental and epileptic encephalopathies (DEEs) due to other etiologies.

Two authors (MT and AF) performed a search in PubMed and EMBASE databases, from 1970 to 2021, using the following keywords: "ceroid lipofuscinosis" and "photoparoxysmal response," "electroencephalogram", "evoked potentials," and "electroretinogram." They looked for experimental and clinical studies and reviews. No meta-analyses were found.

NEUROPHYSIOLOGICAL STUDIES

Electroencephalogram

Epilepsy is one of the cardinal symptoms of NCLs (5). The age at epilepsy onset may differ according to the NCLs variant, starting from the first months of life to adulthood; both focal and generalized seizures can be seen at onset (5). Epilepsy may represent the presenting symptom, as it happens in CLN2 and CLN6 diseases, or appear after visual loss or cognitive and motor decline, as in CLN3 and CLN5 diseases (20).

In the classic form of CLN1 disease, symptoms begin during infancy, and seizure onset is relatively early, typically between the age of 14 and 36 months. Additional phenotypes have been observed with late infantile, juvenile, and adult onset. In the latter cases, epilepsy typically begins several years after the initial symptom of visual impairment, following developmental regression and behavioral changes (20). Few data are available on EEG features in CLN1 disease (21, 22). It has been reported that a slowing of background activity and loss of sleep spindles is associated with high-voltage slow waves and spike and waves abnormalities (22). Between 5 and 12 years of age, a progressive flattening of cerebral activity consistent with the marked cortical atrophy due to massive neuronal death is evident (21). In summary, in CLN1 disease, changes of background activity have been distinguished in three stages: (1) decreasing of reactivity of the posterior rhythm to eye opening and closing; (2) decreasing of sleep spindles and subsequent disappearance; and (3) slowing and/or attenuation of EEG to inactivity, the so-called *vanishing* EEG pattern (22, 23).

In CLN2 disease, a late-infantile neuronal ceroid lipofuscinosis (LINCL), seizures often appear in an explosive fashion (24). Seizures are resistant to common anti-seizure medications (ASMs), and sodium channel blockers, such as carbamazepine, can even worsen seizure frequency and may increase myoclonus and ataxia (24).

The first EEG is usually performed within the fourth year of life and shows a normal background activity, associated with focal or diffuse abnormalities in 75% of the patients. High-voltage focal slow waves are prevalent over the temporal and occipital regions (25–28). Photoparoxysmal response (PPR) to intermittent photic stimulation (IPS) delivered at a low frequency of stimulation is typically seen in patients with CLN2 during the first stage of the disease (27). With disease progression, the background activity becomes slow and without reactivity to eye opening (29, 30). No spindles are present during sleep recordings. Generalized, focal and multifocal epileptiform discharges characterized by irregular spikes and poly-spikes and waves become common; however, a posterior predominance persists (24) (**Figures 1A–E**).

In CLN3 disease, the juvenile neuronal ceroid lipofuscinosis (Spielmeyer–Vogt disease or Batten disease), the presenting manifestation is a progressive visual loss starting around the age of 6–8 years. Epilepsy usually starts at the age of 10 years. Seizures at onset are usually bilateral tonic–clonic, whereas focal seizures increase during adolescence, and they are mostly characterized by clonic manifestations. Myoclonic seizures are rarely reported in CLN3 disease. EEG, in the early stages of the disease, shows focal epileptiform abnormalities (before 10 years of age), whereas bilateral and multifocal epileptiform discharges are significantly more prevalent in the later stages, along with a progressive slowing of the background activity (31). PPR has not been reported as a prominent feature in CLN3 disease (31).

The CLN6 disease may start both during infancy and adulthood. In both cases, epilepsy is one of the presenting symptoms (32). Background activity is poorly organized during awake and sleep since the onset, associated with irregular slow spike and waves discharges (at about 2.5 Hz). PPR to lower frequency is an early neurophysiological finding. During the advanced stage of disease, the cerebral activity became extremely slow, and low-voltage and spike and waves discharge became rare, often replaced by single spikes with multifocal distribution (32).

In adult patients affected by CLN6, the background activity is almost preserved in the early stage of the disease. Epileptiform abnormalities are the most prevalent over the posterior regions. Myoclonus is typically induced by active movements or provoked by IPS at a low frequency of stimulus. Abnormal PPR persists until the advanced stages of the disease (32).

The CLN5 disease, also known as the Finnish variant, starts between the age of 2 and 6 years with clumsiness and mental decline (33, 34). Seizure occurs relatively late (median

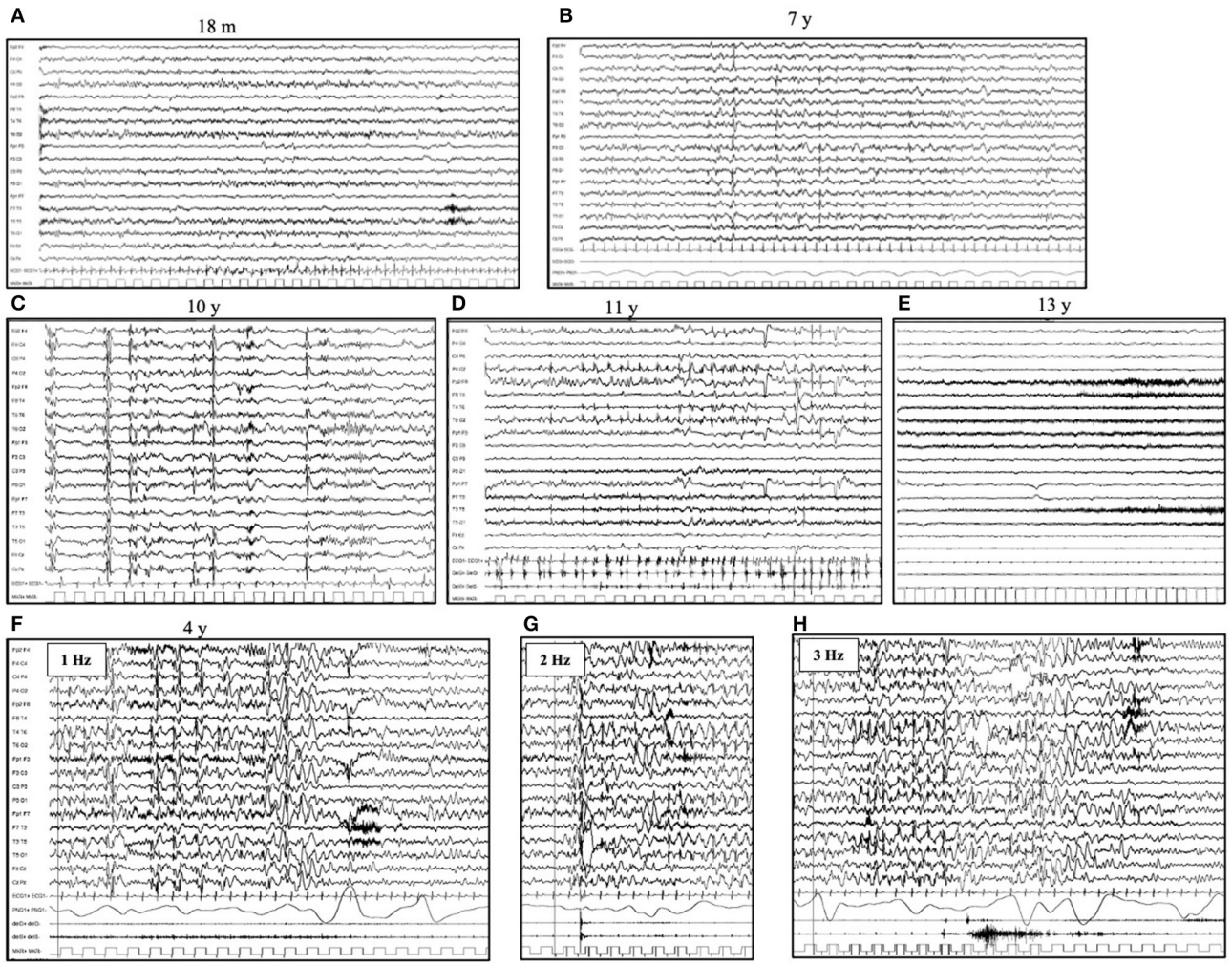

FIGURE 1 | Electroencephalogram (EEG) evolution in CLN2 disease. Awake EEG **(A)** at the age of 18 months (pre-symptomatic phase) shows a normal background activity, with 8 Hz rhythm over bilateral posterior regions. No epileptiform abnormalities are evident. Awake EEG **(B)** at the age of 7 years shows a slow background activity and the lack of regional differentiation. There are diffuse and focal (mainly occipital) spikes and spike and waves abnormalities. During sleep **(C)**, the EEG (age of 10 years) shows the lack of sleep spindles and bursts of rapid spikes and poly-spikes, alternated with the suppression of cerebral activity. **(D)** Myoclonic status can occur in the advanced phases of diseases (age of 11 years) and **(E)** A diffuse flattening of cerebral activity with an extremely slow and low-voltage cerebral activity is typical of the latter stage. **(F–H)** Photoparoxysmal response in a 4-year-old girl affected by CLN2 disease. The flash-per-flash response is evident at the lower frequency of intermittent photic stimulation, at 1, 2, and 3 Hz (respectively **F–H**).

age of 8 years), and epilepsy has its major expression with myoclonic seizures between the age of 7 and 11 years. In addition, for this LINCL, it has been reported that a progressive slowing of background activity is associated with multifocal epileptiform discharges (spikes, multiple spikes, and spike and wave complexes) (34) and the presence of posterior spikes triggered by low-frequency IPS (33, 34).

Regarding CLN7 disease, in few published patients, it has been reported that a progressive generalized slowing of background activity is associated with diffuse or multifocal abnormalities with occipital prevalence (**Figures 2A–C**). PPR has not been reported (35, 36).

In patients with CLN8, it has been described as a progressively slowing of background activity with focal and/or generalized abnormalities since the onset. PPR has rarely been reported (37).

Few cases of CLN11 have been reported (38, 39). EEG is characterized by a quite preserved background activity associated with rare paroxysms of diffuse spikes and slow waves, prevalent over bilateral parietal regions. PPR has not been detected in the three cases reported so far (38, 39).

Limited data have been reported on EEG features for the other NCL variants, from CLN9 to CLN14, which are the more recently identified forms. More cases need to be reported to understand their typical neurophysiological findings.

Photoparoxysmal Response

Photoparoxysmal response in NCLs was firstly described in 1970 when it was reported in about 45% of patients (29).

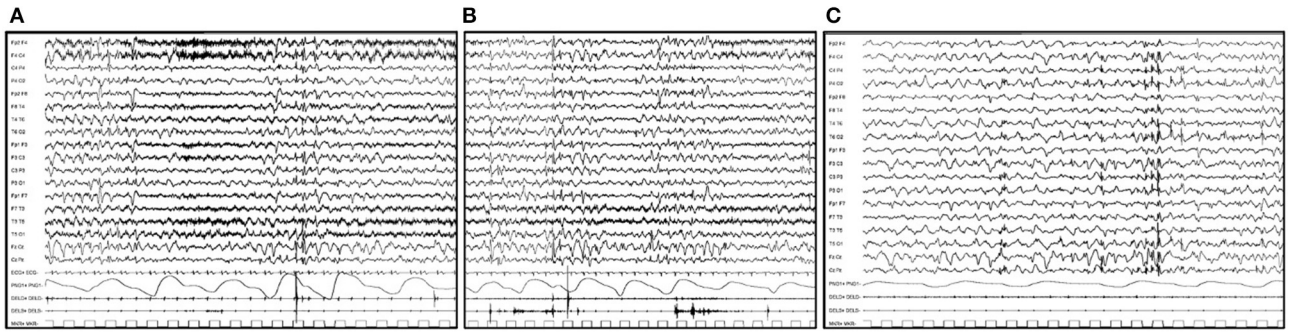

FIGURE 2 | EEG features in a 6-year-old boy affected by CLN7 disease. **(A)** EEG trace shows a generalized slowing of background activity associated with diffuse or multifocal abnormalities. **(B)** Single myoclonic jerks involving the upper limbs with the EEG correlate of a diffuse single spike are evident. **(C)** During sleep, the cerebral activity is markedly slow and is associated with multifocal spike and spike and waves abnormalities, prevalent over bilateral posterior regions.

Later, other studies confirmed the presence of this peculiar neurophysiological finding (27, 32, 40, 41).

The susceptibility to PPR varies accordingly to the different diseases which are more prominent in the LINCLs, such as CLN2, CLN5, and CLN6 (**Figures 1F–H**). In CLN2 disease, PPR has been reported from 27 to 93% of patients (25, 27, 40, 41). This wide range may be explained by the lack of a universal approach of low frequency IPS in routine pediatric EEG recordings (24). In fact, in NCLs, as well as in other progressive myoclonic epilepsies, PPR is one of the earliest pieces of evidence of a neurodegenerative disorder, even before the onset of cognitive and motor regression (42). PPR to low-frequency stimulation has also been reported to occur in Lafora disease and mitochondrial diseases (43, 44).

In a recently reported series of 14 cases of CLN2, serial EEGs revealed a PPR in 93% of patients (27). PPR was evident since the first EEG, which was performed at 3.6 (3.1–4.0) years, in 43% of patients; it was documented at low (1–3 Hz) frequencies of stimulation in 69% of patients and has acquired the form of a flash-per-flash response in 69% of patients (27). Moreover, in the advanced stages of the disease, PPR was associated with massive myoclonic jerks (27). In about half of patients (54%), PPR disappears over time (25). PPR changes over time reflect the gray matter changes due to the progression of neurodegenerative disease (27).

The PPR seen in the NCLs is characterized by an occipital spike and waves response to the photic stimuli. However, it should also be highlighted that not in all NCL variants do patients have the same susceptibility to PPR; it has been reported more frequently in CLN2, CLN5, and CLN6 diseases (24, 27, 32, 33) and seems to not be a prominent feature in CLN1, CLN3, CLN8, and CLN11 diseases (21, 31, 37, 38). For the remaining NCLs, we do not have sufficient data about PPR to find out conclusive data.

On the other hand, the lack of the characteristic IPS response cannot rule out an NCL disease (45). The implementation of IPS, including IPS at low frequencies of stimulation as part of a standard EEG, may be useful as an early disease marker if associated with other clinical findings (24, 45, 46).

Flash and Pattern-Reversal Visual Evoked Potentials

Few data are available in the literature regarding visual evoked potentials (VEPs) changes during the disease course in NCLs. What is known is that with disease progression, the so-called *giant* VEPs appear (**Figures 3D,E**). They are abnormally broad and of high amplitude, and their presence is a marker of cortical hyperexcitability, similar to PPR (32).

Abnormal VEPs have been found in about 75% of patients with CLN2 at a median age of 4.5 years (25). Furthermore, a more recent study on patients with CLN2 evidenced that most patients (89%) had an early and high-amplitude pattern reversal in VEPs, whereas few patients showed a bifid waveform, which is associated with a central scotoma, indicative of maculopathy or macular pathway dysfunction such as optic atrophy (47).

With further disease progression, the amplitude of VEP decreases, as it happens in other neurodegenerative disorders (48, 49).

The pattern-reversal VEP waveform is preferred to the flash VEP waveform, which shows a wide inter-individual variability. On the contrary, the pattern-reversal VEP has a relatively constant single positive peak throughout life and is a strong index of macular pathway function (47).

Variability of results may be due to the retrospective nature of the studies and the different ages and phases of the disease in which visual tests have been performed (25, 41, 47).

Electroretinogram

Vision-related problems are one of the cardinal signs of NCLs and, as in CLN3, are often an early sign, appearing prior to motor and mental deterioration (5). This is due to the accumulation of storage material into the retina, leading to its degeneration (50, 51).

Electroretinogram (ERG) represents the tool that allows to monitor the involvement of retina, and its use progressively disappears with disease progression (**Figures 3A–C**) at different ages according to the NCL variants (50). ERG is currently used to

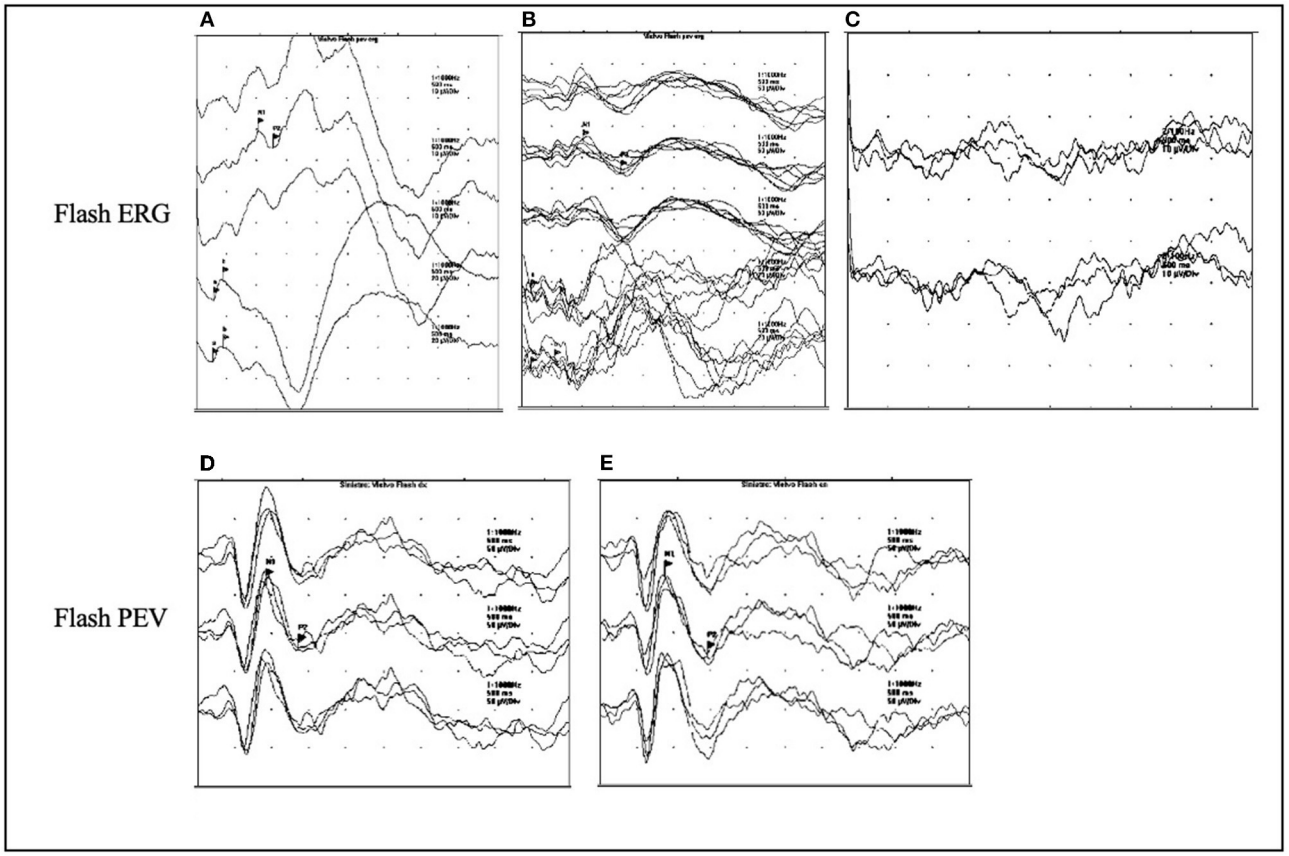

FIGURE 3 | ERG and VEPs in CLN2 disease. **(A–C)** The ERG flash evolution in a patient affected with CLN2 at the ages of 3.5, 5.5, and 7 years. The response is normal at the onset of disease at the age of 3.5 years **(A)**, at the age of 5.5 years, there is a reduction of the amplitude of ERG **(B)**, which turns to be absent at the age of 7 years **(C)**. **(D,E)** The presence of giant flash VEPs in the left and right eyes in a 4-year-old patient.

characterize the physiological changes in the degenerating retina in patients affected by NCLs. It allows to identify the retinopathy, which consists of symmetrical cone-rod dystrophy (47, 50).

In patients with CLN2, it has been demonstrated that changes in ERG appear even before the macular disruption on optical coherence tomography (OCT) from the age of 4 years and 10 months (47). Abnormal ERG has been reported in 66% of patients at a median age of 4.5 years (25). However, it is important to underlie that the flattening of ERG does not necessarily imply the total loss of retinal function, if some cortical VEP functions persist (29).

In CLN5 disease, retinal degeneration has been confirmed between the age of 6 and 10 years, when ERG has been found abolished in most of the patients (33, 34).

The use of ERG might be implemented in the future to evaluate the efficacy of experimental treatments with intravitreal therapies.

Somatosensory Evoked Potentials

Somatosensory evoked potentials (SEPs) have been poorly investigated in NCL diseases. There are sporadic cases, across different forms of NCLs (21, 32, 33, 52), where it is highlighted that the presence of high-amplitude evoked potential, *giant* SEPs, which are the expressions of cortical hyperexcitability due to neuronal degeneration (32).

Giant SEPs are also a typical and specific marker of patients with cortical myoclonus, which is one of the main clinical features of NCLs (38).

DIFFERENTIAL DIAGNOSIS

Neurophysiological investigations can also be useful to differentiate NCLs from other DEEs. In detail, LINCLs should be differentiated from those DEEs with epilepsy onset between 2 and 6 years of age, mainly characterized by myoclonic seizures. Among these, it is important to consider epilepsy with myoclonic-atonic seizures (EMA) and Lennox–Gastaut syndrome (LGS), which are characterized by epilepsy with multiple types of seizures, intellectual disability, and drug resistance (24).

The EEG is particularly useful for this purpose because, in NCLs, there is a slowing of background activity with progressive loss of differentiation and disappearance of spindles during sleep, whereas in EMA, although with the presence of many epileptiform abnormalities, the background activity is preserved (53). Moreover, a poly-graphic study might highlight that, in

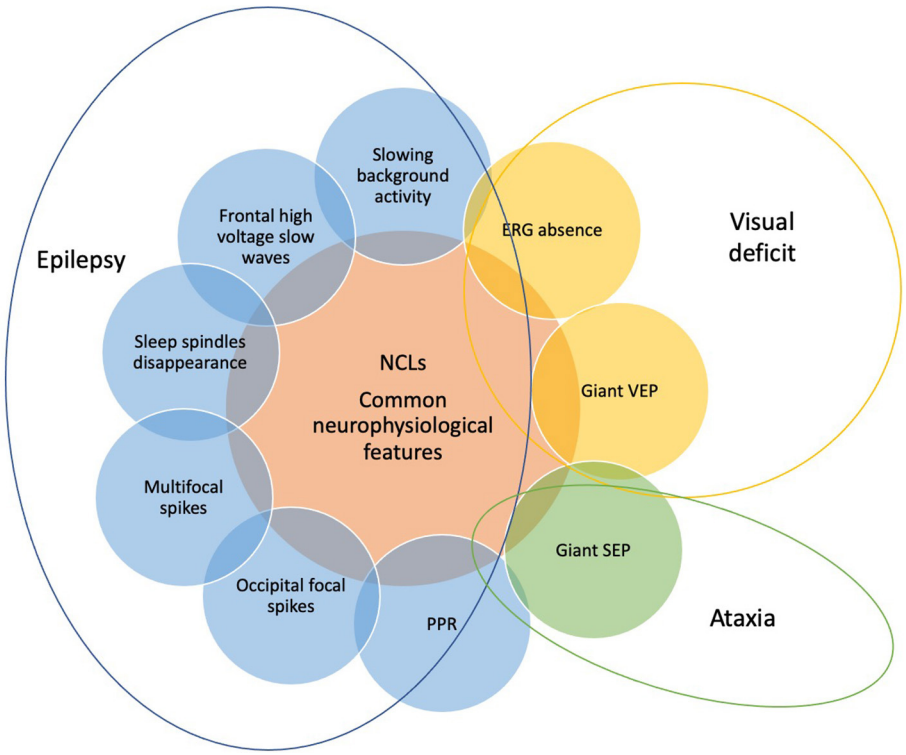

FIGURE 4 | The common neurophysiological features of NCLs include EEG, visual evoked potentials (VEPs), somatosensory evoked potentials (SEPs), and electroretinogram findings. They can vary according to the different subtypes of NCLs and the age of patients. Each neurophysiological finding (slowing background activity, disappearance of sleep spindles, frontal high-voltage slow waves, occipital focal spikes, multifocal spikes, PPR, giant VEPs and SEP, and ERG absence) is the expression of one of the three main symptoms of NCLs, which are epilepsy, visual deficit, and ataxia and allows to monitor their progression.

EMA, myoclonic events mainly involve proximal, axial muscles, whereas, in NCLs, myoclonus involves mainly distal, segmental muscles (38, 53).

The LGS is otherwise characterized by specific EEG abnormalities, which are diffuse spike-and-slow wave complexes at 2.5 Hz during awake, and poly-spikes during sleep (54). In both syndromes, PPR is rarely reported and, when described, it is not induced by low-frequency IPS (53, 54).

On the other hand, PPR is reported in some epileptic syndromes of infancy, and among these, The Dravet syndrome (DS) is most frequent (55). Different from the majority of NCLs, in DS, epilepsy starts within the first year of life. In addition, in DS, PPR is not typically induced by low-frequency IPS and does not have the no characteristics of the flash-per-flash PPR (55).

During the last decades, technological advances have driven genetic discovery in epilepsy and increased the understanding of the molecular mechanisms of many epileptic disorders, in some cases providing targets for precision medicine (19, 56). Nevertheless, phenotyping and neurophysiological characterization are still critical for the diagnosis and better management of neurological symptoms.

CONCLUSIONS

An appropriate definition of neurophysiological features of NCLs is crucial for the possible role that they may have in the early diagnosis of such diseases. There are some features (**Figure 4**) that are common to different subtypes of NCLs, such as the progressive slowing of background activity, the disappearance of sleep spindles during sleep, and the presence of some type of heterogeneous abnormalities, such as bursts of diffuse or focal slow waves prevalent over temporal and occipital regions and diffuse spike and wave paroxysmal discharges (25–27).

Sensitivity to low-frequency IPS is a hallmark of neurodegenerative diseases such as NCLs and may be useful as an early marker if associated with other clinical findings (27). This peculiar EEG activation may be missed due to the lack of standardization of the test, which should be implemented with low-frequency stimulation, starting from 1 Hz (27).

The VEPs allow to monitor the visual pathway function, and the lack of visual response at ERG reflects retinal neurodegeneration.

Taken together, EEG, VEPs, and ERG may represent essential tools that can address the clinicians toward an early diagnosis of NCL disease. Although treatment remains essentially symptomatic in NCLs, together with palliative, supportive, and rehabilitative measures, in the near future, ERT and gene therapies may be available and earlier diagnosis will be mandatory. In this context, the use of NGS-based approaches results important for the early identification of patients with NCL, allowing a timely adoption of the most accurate treatment strategies.

AUTHOR CONTRIBUTIONS

MT and AF prepared the first draft of the manuscript. NS and FV coordinated the job and supervised critically the manuscript and reviewed the final version of the manuscript. CC did the reference search and provided support for the discussion. LP and GC prepared the figures and contributed to the figure legends and reviewed critically the manuscript. NP reviewed critically the manuscript and contributed to the final draft. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Platt FM. Emptying the stores: lysosomal diseases and therapeutic strategies. *Nat Rev Drug Discov.* (2018) 17:133–50. doi: 10.1038/nrd.2017.214
2. Nelvagal HR, Lange J, Takahashi K, Tarczyluk-Wells MA, Cooper JD. Pathomechanisms in the neuronal ceroid lipofuscinoses. *Biochim Biophys Acta Mol Basis Dis.* (2019) 31:165570. doi: 10.1016/j.bbadis.2019.165570
3. Rakheja D, Bennett MJ. Neuronal ceroid-lipofuscinoses. *Transl Sci Rare Dis.* (2018) 3:83–95. doi: 10.3233/TRD-180024
4. Glees P, Hasan M. Lipofuscin in neuronal aging and diseases. *Norm Pathol Anat (Stuttg).* (1976) 32:1–68.
5. Schulz A, Kohlschütter A, Mink J, Simonati A, Williams R. NCL diseases – clinical perspectives. *Biochim Biophys Acta.* (2013) 1832:1801–6. doi: 10.1016/j.bbadis.2013.04.008
6. Sondhi D, Crystal RG, Kaminsky SM. Gene Therapy for Inborn Errors of Metabolism: Batten Disease. In: Tuszynski MH, editor. *Translational Neuroscience: Fundamental Approaches for Neurological Disorders*. Boston, MA: Springer US (2016). p. II 1–129.
7. Mole SE, Anderson G, Band HA, Berkovic SF, Cooper JD, Kleine Holthaus SM, et al. Clinical challenges and future therapeutic approaches for neuronal ceroid lipofuscinosis. *Lancet Neurol.* (2019) 18:107–16. doi: 10.1016/S1474-4422(18)30368-5
8. Johnson TB, Cain JT, White KA, Ramirez-Montealegre D, Pearce DA, Weimer JM. Therapeutic landscape for Batten disease: current treatments and future prospects. *Nat Rev Neurol.* (2019) 15:161–78. doi: 10.1038/s41582-019-0138-8
9. Mole SE, Haltia M. Chapter 70 - The Neuronal Ceroid-Lipofuscinoses (Batten Disease). In: Rosenberg RN, Pascual JM, editors. *Rosenberg's Molecular and Genetic Basis of Neurological and Psychiatric Disease (Fifth Edition)*. Boston: Academic Press (2015). p. 793–808. doi: 10.1016/B978-0-12-410529-4.00070-X
10. Nita DA, Mole SE, Minassian BA. Neuronal ceroid lipofuscinoses. *Epileptic Disord.* (2016) 18:73–88. doi: 10.1684/epd.2016.0844
11. Donsante A, Boulis NM. Progress in gene and cell therapies for the neuronal ceroid lipofuscinoses. *Expert Opin Biol Ther.* (2018) 18:755–64. doi: 10.1080/14712598.2018.1492544
12. Schulz A, Ajayi T, Specchio N, de Los Reyes E, Gissen P, Ballon D, et al. CLN2 Study Group. Study of Intraventricular Cerliponase Alfa for CLN2 Disease. *N Engl J Med.* (2018) 378:1898–907. doi: 10.1056/NEJMoa1712649
13. Specchio N, Ferretti A, Trivisano M, Pietrafusa N, Pepi C, Calabrese C, et al. Neuronal ceroid lipofuscinosis: potential for targeted therapy. *Drugs.* (2021) 81:101–23. doi: 10.1007/s40265-020-01440-7
14. Desnick RJ, Schuchman EH. Enzyme replacement therapy for lysosomal diseases: lessons from 20 years of experience and remaining challenges. *Annu Rev Genomics Hum Genet.* (2012) 13:307–35. doi: 10.1146/annurev-genom-090711-163739
15. Solomon M, Muro S. Lysosomal enzyme replacement therapies: Historical development, clinical outcomes, and future perspectives. *Adv Drug Deliv Rev.* (2017) 118:109–34. doi: 10.1016/j.addr.2017.05.004
16. Kruer MC, Pearce DA, Orchard PJ, Steiner RD. Prospects for stem cell therapy in neuronal ceroid lipofuscinosis. *Regen Med.* (2013) 8:527–9. doi: 10.2217/rme.13.46
17. Nickel M, Simonati A, Jacoby D, Lezius S, Kilian D, Vande GraafB, et al. Disease characteristics and progression in patients with late-infantile neuronal ceroid lipofuscinosis type 2 (CLN2) disease: an observational cohort study [published correction appears in Lancet Child Adolesc Health. 2018 2:e24]. *Lancet Child Adolesc Health.* (2018) 2:582–90. doi: 10.1016/S2352-4642(18)30179-2
18. Fietz M, AlSayed M, Burke D, Cohen-Pfeffer J, Cooper JD, Dvoráková L, et al. Diagnosis of neuronal ceroid lipofuscinosis type 2 (CLN2 disease): Expert recommendations for early detection and laboratory diagnosis. *Mol Genet Metab.* (2016) 119:160–7. doi: 10.1016/j.ymgme.2016.07.011
19. Amadori E, Scala M, Cereda GS, Vari MS, Marchese F, Di Pisa V, et al. Targeted re-sequencing for early diagnosis of genetic causes of childhood epilepsy: the Italian experience from the 'beyond epilepsy' project. *Ital J Pediatr.* (2020) 46:92. doi: 10.1186/s13052-020-00860-1
20. Augustine EF, Adams HR, de Los Reyes E, Drago K, Frazier M, Guelbert N, et al. Management of CLN1 disease: international clinical consensus. *Pediatr Neurol.* (2021) 120:38–51. doi: 10.1016/j.pediatrneurol.2021.04.002
21. Simonati A, Tessa A, Bernardina BD, Biancheri R, Veneselli E, Tozzi G, et al. Variant late infantile neuronal ceroid lipofuscinosis because of CLN1 mutations. *Pediatr Neurol.* (2009) 40:271–6. doi: 10.1016/j.pediatrneurol.2008.10.018
22. Vanhanen SL, Sainio K, Lappi M, Santavuori P. (1997) EEG and evoked potentials in infantile ceroid-lipofuscinosis. Dev Med Child Neurol 39:456-463Jalanko A, Braulke T Neuronal ceroid lipofuscinoses. *Biochim Biophys Acta.* (2009) 1793:697–709. doi: 10.1016/j.bbamcr.2008.11.004
23. Veneselli E, Biancheri R, Buoni S, Fois A. Clinical and EEG findings in 18 cases of late infantile neuronal ceroid lipofuscinosis. *Brain Dev.* (2001) 23:306–11. doi: 10.1016/S0387-7604(01)00231-5
24. Johnson AM, Mandelstam S, Andrews I, Boysen K, Yaplito-Lee J, Fietz M, et al. Neuronal ceroid lipofuscinosis type 2: an Australian case series. *J Paediatr Child Health.* (2020) 56:1210–8. doi: 10.1111/jpc.14890
25. Beltrán L, Valenzuela GR, Loos M, Vargas R, Lizama R, Spinsanti P, et al. Late-onset childhood neuronal ceroid lipofuscinosis: early clinical and electroencephalographic markers. *Epilepsy Res.* (2018) 144:49–52. doi: 10.1016/j.eplepsyres.2018.05.005
26. Binelli S, Canafoglia L, Panzica F, Pozzi A, Franceschetti A. Electroencephalographic features in a series of patients with neuronal ceroid lipofuscinoses. *Neurol Sci.* (2000) 21:S83. doi: 10.1007/s100720070045
27. Specchio N, Bellusci M, Pietrafusa N, Trivisano M, de Palma L, Vigevano F. Photosensitivity is an early marker of neuronal ceroid lipofuscinosis type 2 disease. *Epilepsia.* (2017) 58:1380–8. doi: 10.1111/epi.13820
28. Veneselli E, Biancheri R, Perrone MV, Buoni S, Fois A. Neuronal ceroid lipofuscinoses: clinical and EEG findings in a large study of Italian cases. *Neurol Sci.* (2000) 21(3 Suppl):S75-81. doi: 10.1007/s100720070044
29. Pampiglione G, Harden A. So-called neuronal ceroid lipofuscinosis. *J Neurol Neurosurg Psychiatry.* (1977) 40:323–30. doi: 10.1136/jnnp.40.4.323
30. Aicardi J, Plouin P, GoutieÂres F. Ceroid-lipofuscinoses. *Rev EEG Neurophysiol.* (1978) 8:149±159. doi: 10.1016/S0370-4475(78)80130-0
31. Arntsen V, Strandheim J, Helland IB, Sand T, Brodtkorb E. Epileptological aspects of juvenile neuronal ceroid lipofuscinosis (CLN3 disease) through the lifespan. *Epilepsy Behav.* (2019) 94:59–64. doi: 10.1016/j.yebeh.2019.02.020
32. Canafoglia L, Gilioli I, Invernizzi F, Sofia V, Fugnanesi V, Morbin M, et al. Electroclinical spectrum of the neuronal ceroid lipofuscinoses associated with CLN6 mutations. *Neurology.* (2015) 85:316–24. doi: 10.1212/WNL.0000000000001784
33. Holmberg V, Lauronen L, Autti T, Santavuori P, Savukoski M, Uvebrant P, et al. Phenotype-genotype correlation in eight patients with Finnish variant late infantile NCL (CLN5). *Neurology.* (2000) 55:579–81. doi: 10.1212/WNL.55.4.579
34. Simonati A, Williams RE, Nardocci N, Laine M, Battini R, Schulz A, et al. Phenotype and natural history of variant late infantile ceroid-lipofuscinosis 5. *Dev Med Child Neurol.* (2017) 59:815–21. doi: 10.1111/dmcn.13473
35. Aiello C, Terracciano A, Simonati A, Discepoli G, Cannelli N, Claps D, et al. Mutations in MFSD8/CLN7 are a frequent cause of variant-late infantile neuronal ceroid lipofuscinosis. *Hum Mutat.* (2009) 30:E530–40. doi: 10.1002/humu.20975

36. Craiu D, Dragostin O, Dica A, Hoffman-Zacharska D, Gos M, Bastian AE, et al. Rett-like onset in late-infantile neuronal ceroid lipofuscinosis (CLN7) caused by compound heterozygous mutation in the MFSD8 gene and review of the literature data on clinical onset signs. *Eur J Paediatr Neurol.* (2015) 19:78–86. doi: 10.1016/j.ejpn.2014.07.008
37. Striano P, Specchio N, Biancheri R, Cannelli N, Simonati A, Cassandrini D, et al. Clinical and electrophysiological features of epilepsy in Italian patients with CLN8 mutations. *Epilepsy Behav.* (2007) 10:187–91. doi: 10.1016/j.yebeh.2006.10.009
38. Canafoglia L, Morbin M, Scaioli V, Pareyson D, D'Incerti L, Fugnanesi V, et al. Recurrent generalized seizures, visual loss, and palinopsia as phenotypic features of neuronal ceroid lipofuscinosis due to progranulin gene mutation. *Epilepsia.* (2014) 55:e56–9. doi: 10.1111/epi.12632
39. Kamate M, Detroja M, Hattiholi V. Neuronal ceroid lipofuscinosis type-11 in an adolescent. *Brain Dev.* (2019) 41:542–5. doi: 10.1016/j.braindev.2019.03.004
40. Albert DV, Yin H, De Los Reyes EC, Vidaurre J. Unique Characteristics of the Photoparoxysmal Response in Patients With Neuronal Ceroid Lipofuscinosis Type 2: Can EEG Be a Biomarker? *J Child Neurol.* (2016) 31:1475–82. doi: 10.1177/0883073816658659
41. Dozières-Puyravel B, Nasser H, Elmaleh-Bergès M, Lopez Hernandez E, Gelot A, Ilea A, et al. Paediatric-onset neuronal ceroid lipofuscinosis: first symptoms and presentation at diagnosis. *Dev Med Child Neurol.* (2020) 62:528–30. doi: 10.1111/dmcn.14346
42. Kasteleijn-Nolst Trenité D, Mastrangelo M, Spalice A, Parisi P. Photosensitivity as an early marker of epileptic and developmental encephalopathies. *Epilepsia.* (2018) 59:1086–7. doi: 10.1111/epi.14031
43. Roger J, Pellissier JF, Bureau M, Dravet C, Revol M, Tinuper P. Early diagnosis of Lafora disease: significance of paroxysmal visual manifestations and contribution of skin biopsy. *Rev Neurol (Paris).* (1983) 139:115–24.
44. Canafoglia L, Franceschetti S, Antozzi C, et al. Epileptic phenotypes associated with mitochondrial disorders. Neurology. (2001) 56:1340–6. doi: 10.1212/WNL.56.10.1340
45. Trivisano M, Specchio N. Red flags for neuronal ceroid lipofuscinosis type 2 disease. *Dev Med Child Neurol.* (2020) 62:414. doi: 10.1111/dmcn.14389
46. Nickel M, Jacoby D, Lezius S, Down M, Simonati A, Genter F, et al. Natural history of CLN2 disease: quantitative assessment of disease characteristics and rate of progression. *Neuropediatrics.* (2016) 47 (FV04-03). doi: 10.1055/s-0036-1583730
47. Thompson DA, Handley SE, Henderson RH, Marmoy OR, Gissen P. An ERG and OCT study of neuronal ceroid lipofuscinosis CLN2 Battens retinopathy. *Eye (Lond).* (2021) 35:2438–48. doi: 10.1038/s41433-021-01594-y
48. Sainio K. Neurophysiological findings in neuronal ceroid lipofuscinoses. *Neuropediatrics.* (1997) 8:70. doi: 10.1055/s-2007-973673
49. Kobayashi K, Hitomi T, Matsumoto R, Kondo T, Kawamata J, Matsuhashi M, et al. Long-term follow-up of cortical hyperexcitability in Japanese Unverricht-Lundborg disease. *Seizure.* (2014) 23:746–50. doi: 10.1016/j.seizure.2014.06.002
50. Quagliato EMAB, Rocha DM, Sacai PY, Watanabe SS, Salom∼ao SR, Berezovsky A. Retinal function in patients with the neuronal ceroid lipofuscinosis phenotype. *Arq Bras Oftalmol.* (2017) 80:215–9. doi: 10.5935/0004-2749.20170053
51. Goebel HH, Klein H, Santavuori P, Sainio K. Ultrastructural studies of the retina in infantile neuronal ceroid-lipofuscinosis. *Retina.* (1988) 8:59–66. doi: 10.1097/00006982-198808010-00011
52. Jadav RH, Sinha S, Yasha TC, Aravinda H, Gayathri N, Rao S, et al. Clinical, electrophysiological, imaging, and ultrastructural description in 68 patients with neuronal ceroid lipofuscinoses and its subtypes. *Pediatr Neurol.* (2014) 50:85–95. doi: 10.1016/j.pediatrneurol.2013.08.008
53. Trivisano M, Specchio N, Cappelletti S, Di Ciommo V, Claps D, Specchio LM, et al. Myoclonic astatic epilepsy: an age-dependent epileptic syndrome with favorable seizure outcome but variable cognitive evolution. *Epilepsy Res.* (2011) 97:133–41. doi: 10.1016/j.eplepsyres.2011.07.021
54. Berg AT, Levy SR, Testa FM. Evolution and course of early life developmental encephalopathic epilepsies: Focus on Lennox-Gastaut syndrome. *Epilepsia.* (2018) 59:2096–105. doi: 10.1111/epi.14569
55. Specchio N, Pontrelli G, Serino D, Trivisano M, Cappelletti S, Terracciano A, et al. Occipital seizures induced by intermittent photic stimulation in Dravet syndrome. *Seizure.* (2014) 23:309–13. doi: 10.1016/j.seizure.2013.12.009
56. Balestrini S, Chiarello D, Gogou M, Silvennoinen K, Puvirajasinghe C, Jones WD, et al. Real-life survey of pitfalls and successes of precision medicine in genetic epilepsies. *J Neurol Neurosurg Psychiatry.* (2021) 92:1044–52. doi: 10.1136/jnnp-2020-325932

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Pacemaker Implantation in Juvenile Neuronal Ceroid Lipofuscinosis (CLN3)–A Long-Term Follow-Up Study

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It is well documented that deteriorating heart function due to deposition of ceroid lipopigment is a significant co-morbidity in Juvenile Neuronal Ceroid Lipofuscinosis (CLN3 disease) although the exact disease mechanisms remain unknown in any NCL form. An increasing frequency of cardiac conduction disorders including severe bradycardia and sinus arrest is seen in the late teens, as is a left ventricular hypertrophy in the early 20s. Only a few case reports of pacemaker implantation have been published, and so far, no long-term follow-up study exists. As new treatment options emerge, more patients will live longer and the need for pacemaker will likely increase, why knowledge of long-term outcome is needed. In the present study, we present the course of six patients from the original Danish CLN3-heart population study ($n = 29$) published in 2011 in whom pacemaker implantation was indicated from a cardiac point of view. In two cases, the families deselected pacemaker implantation. In four males, aged 19-29 years, all having a good general condition, a dual-chamber pacemaker (St. Jude Medical™ Accent/Assurity MRI™) was implanted in general anesthesia without any complications. At follow-up 9 years later, three were still alive. According to the parents' opinion they still have a good quality of life, now 26, 30, and 36 years old. Pacemaker treatment is safe and may have great impact on quality of life. However, the medical indication for pacemaker treatment is relative and it is important that various aspects, including the patient's general condition and family preferences, are thoroughly discussed before making the final decision.

Keywords: Juvenile Neuronal Ceroid Lipofuscinosis, CLN3, neurodegenerative diseases, sick sinus syndrome, pacemaker

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INTRODUCTION

The Neuronal Ceroid Lipofuscinoses (NCL) is a group of hereditary diseases characterized by dysfunction of the lysosomes. Over time, increasing deposition of ceroid lipopigment leads to increasing degeneration and loss of function of the cells in question. There are today 14 different subtypes that all but one debut in childhood.

The Juvenile Neuronal Ceroid Lipofuscinosis, also called CLN3 disease, is a fatal neurodegenerative disease with an estimated incidence range from 0.2 to 7.0 per 100,000 (1). It is an autosomal recessive disorder caused by a defect in the *CLN3* gene, which in the majority of

cases (>85%) is due to a homozygous deletion of exon 7/8 (2). The disease initially presents with visual impairments and within a few years, decline of cognitive and motor function, behavioral changes and epilepsy follow. As the disease initially presents with degenerative changes of the nervous system, therapeutics and research have mainly been concentrated concerning pathology changes of the CNS, but in recent years it has been well documented, that other organ systems, such as the heart, are also affected (3–6). Accumulation of storage material has been reported in CLN3 disease, but knowledge of the exact disease mechanisms in any NCL form is still lacking (3). In 2011, a Danish clinical cross-sectional and follow-up study was published comprising 29 patients with CLN3 (7). In this study, progressive cardiac impairment including repolarization disturbances, ventricular hypertrophy and sinus node dysfunction leading to severe bradycardia and other conduction abnormalities were reported. Inverted T waves were present from 14 years of age and were associated with an increased risk of early death. Bradycardia and left ventricular hypertrophy were found in all patients > 20 years old, and in one patient pacemaker treatment was indicated from a cardiologic point of view and in question. So far, pacemaker implantation in CLN3 disease has only been reported in case studies (4, 5), and no long-term follow-up studies exist. As new treatment options emerge, including gene therapy (8, 9), CLN3 patients may live longer and the need for pacemaker will likely increase. Thus, knowledge of long-term outcome following pacemaker implantation is needed.

In the present follow-up study of the original Danish CLN3 heart population (7), we describe indications, reflections and course of six patients having a severe reduction in heart rate, long periods of sinus arrests and gradually accompanying clinical symptoms of cardiac failure and/or sudden loss of consciousness and muscular tone different from their habitual seizures.

MATERIALS AND METHODS

Participants

For more than 20 years, all Danish patients with CLN3 disease have been regularly treated at one national site, i.e., Centre for Rare Diseases, Aarhus University Hospital. The Centre is tax financed and monitors patients of all types of NCL from infancy until death, regardless of severity of disease and socioeconomic background. In 2003-2010, 29 of 30 patients with CLN3 in Denmark participated in a cross-sectional and follow-up study where 24 h Holter examination, a 12-lead ECG and echocardiography were performed every second year. The study was published in 2011 (7). Since then, all patients < 18 years of age having a reduced minimum heart rate (<40 beats per min) or a reduced mean heart rate (<55 beats per min), and patients above 18 years of age regardless of heart rate, have been offered a yearly 24 h Holter examination. In the present study we included those patients who were eligible for pacemaker implantation if they fulfilled the following criteria:

1) Clinical symptoms of cardiac failure (shortness of breath, severe fatigue, peripheral cyanosis and/or syncope/near-syncope) and SA node dysfunction.

2) A 24 h Holter examination demonstrated severe bradycardia and/or periods of prolonged sinus arrest, which were considered abnormal and explanatory for the above clinical symptoms.

In all patients an echocardiography was performed. In patients who fulfilled the above criteria and where caregivers after thorough information agreed to the procedure, a dual chamber pacemaker (St. Jude Medical™ Accent/Assurity MRI™) was subsequently implanted in general anesthesia.

The present study was conducted as a follow-up study based on both prospective and retrospective, descriptive data. A medical chart review was performed, and data was collected for each patient. The data included information about genetics, demographic characteristics, sex, age and clinical status at the time of assessment for 24 h Holter examination, echocardiography, and pacemaker implantation. Patients were followed until end of study period (August 2021) or death.

Standard 12-Lead ECG Recording

Below 12 years, negative T waves in lead V2 and V3 were considered normal. Above 12 years, the standard criteria for normality were applied. Abnormal findings included complete right bundle branch block, left ventricular hypertrophy, flat or deep negative T waves in V2–V6, pathologic Q waves in lead III, grade I atrioventricular (AV) block, grade II type II AV block, and permanent atrial fibrillation or permanent atrial flutter.

Twenty-Four-Hour Ambulatory ECG Recording (Holter Examination)

To exclude periods with artifacts, each recording was analyzed interactively by an experienced technician. Heart rate (HR) was measured as HR per 1-min period [beats per minute (bpm)]. Maximum HR ($HR_{max,24h}$) and minimum HR ($HR_{min,24h}$) were identified, and mean 24-h HR ($HR_{mean,24h}$) was calculated. Based on values for 95% confidence limits in healthy individuals normal values for $HR_{min,24h}$ and $HR_{mean,24h}$ were set as 40 and 55 bpm, respectively (10).

Echocardiography

Reviewing the ECG's and Holter investigations, and making the assessment of whether or not a pacemaker implantation was indicated, was performed by the same cardiologic specialist (HM) in all the patients. Echocardiography was performed by one of three senior experienced cardiologists on a Vingmed Vivid 7 (GE Healthcare, Oslo, Norway) apparatus using a standardized protocol for evaluation of morphology. Left ventricular hypertrophy was defined as 11-mm wall thickness.

Statistical Analyses

The study was designed as a descriptive study and no statistical analyses were performed.

RESULTS

Six patients (five males) fulfilled the criteria for pacemaker implantation (**Table 1**). The patients were between 19 and 27 years old and all had a homozygous deletion of exon 7/8 in

TABLE 1 | Clinical status at time of implantation and follow-up.

Clinical status at pacemaker inclusion								
Patient number (Sex)	**Genotype**	**Age at pacemaker implantation**	**HR mean (24 h)**	**HR min**	**ECG findings**	**Echocardiography**	**Cardiac-related symptoms**	**Clinical function**
1 (Male)	*CLN3* del (exon 7/8)	20	36	28	Sinus arrhythmia, Sinus arrest	Normal	Syncope Cold hands and feet Peripheral cyanosis	Walk independently Talk in sentences
2 (Male)	*CLN3* del (exon 7/8)	19	54	32	AV block, type 2, Sinus-arrest	Normal	Dyspnea during activity	Walk independently Talk in sentences
3 (Male)	*CLN3* del (exon 7/8)	27	38	28	Sinus-arrest	Ventricular hypertrophy	Cold hands and feet Peripheral cyanosis	Use of wheelchair Use single words No sentences
4 (Male)	*CLN3* del (exon 7/8)	21	45	30	Sinus arrest, 11 s	Not available	Tired Dyspnea	Walk independently Talk in sentences
5 (Male)	*CLN3* del (exon 7/8)	Pacemaker deselected by parents 23 years of age	37	24	Sinus arrest, 21 s	Ventricular hypertrophy	Many syncope's Very tired Peripheral cyanosis	Use of wheelchair Use single words
6 (Female)	*CLN3* del (exon 7/8)	Pacemaker deselected due to severe general condition 22 years of age	53	20	Sinus arrest, 26 s	Normal	Many syncope's Very tired Cold hands and feet Peripheral cyanosis	Use of wheelchair No words, only sounds
Clinical status at follow-up								
Patient number (Sex)	**Genotype**	**Age at follow-up**	**Follow-up (years)**	**Alive**	**Course of death**	**Echocardiography**	**Cardiac-related symptoms**	**Clinical function**
1 (Male)	*CLN3* del (exon 7/8)	30	9	Yes	NA	Ventricular hypertrophy	None	Use of wheelchair No words, only sounds
2 (Male)	*CLN3* del (exon 7/8)	26	6	Yes	NA	Ventricular hypertrophy	None	Use of wheelchair Use single words
3 (Male)	*CLN3* del (exon 7/8)	36	8	Yes	NA	Ventricular hypertrophy	None	Use of wheelchair No words, only sounds
4 (Male)	*CLN3* del (exon 7/8)	passed away at the age of 22	1	No	pneumonia	-	-	-
5 (Male)	*CLN3* del (exon 7/8)	passed away at the age of 25	2	No	pneumonia	-	-	-
6 (Female)	*CLN3* del (exon 7/8)	passed away at the age of 22	0.4	No	pneumonia	-	-	-

CLN3. In two cases pacemaker implantation was omitted. In one case, the procedure was refrained due to a severely affected general condition of the patient and in the second case because the parents were opposed to a possible life-prolonging treatment. All patients had clinical symptoms at the time of the pacemaker implantation. Two patients, who were still physical active, complained shortness of breath and feeling extremely tired. In the other patients, syncope and near-syncope was the primary symptom. In all patients severe sinus bradycardia (**Figure 1**) was demonstrated ($HR_{min,24h}$ between 20 and 32 beats per min), as were prolonged sinus arrests (**Figure 2**). Echocardiography was initially performed in four out of six patients. In two patients, aged 19 and 21 years, a normal examination was found. In the two other patients, aged 23 and 27 years, ventricular hypertrophy was demonstrated. **Figure 3** shows 24 h heart rate profiles before (A) and after (B) pacemaker implantation in one of the treated patients. $HR_{min,24h}$ has increases from 30 to 59 bpm and $HR_{mean,24h}$ from 41 to 60 bpm.

The patients were followed for 9 years or until they deceased. One patient died 1 year after the pacemaker implantation due to pneumonia. The other three patients were still alive and, respectively, 26, 30, and 36 years old at the end of the study period. The patients' caregivers have reported that complaints of shortness of breath, when still physical active disappeared after the pacemaker implantation, and there had been no episodes of syncope in any of the patients. They all got warm extremities following the implantation, and there were no pacemaker related infections or need for re-implantation. At follow-up, all surviving patients had ventricular hypertrophy demonstrated by ultrasound of the heart.

In one case the pacemaker implantation was omitted due to a severe general condition of the patient including intractable epilepsy. The general condition of the patient further deteriorated during the following months and the patient continued to have multiple episodes of sinus arrest of up to 26 s leading to multiple syncope fainting. Four months later the patient died of pneumonia. In the case where pacemaker implantation was omitted because the parents were opposed to a possible life-prolonging treatment, the patients died 2 years later due to pneumonia. In the meanwhile, the patient had recurrent syncope fainting, and repeated ECG's demonstrated multiple episodes of sinus arrest with increasing duration up to 21 s. Echocardiography demonstrated ventricular hypertrophy.

DISCUSSION

In CLN3, pronounced deposition of ceroid lipopigment has been shown to occur in the sinus node of the heart, its autonomic nerve supply, as well as in the atrioventricular node and in the

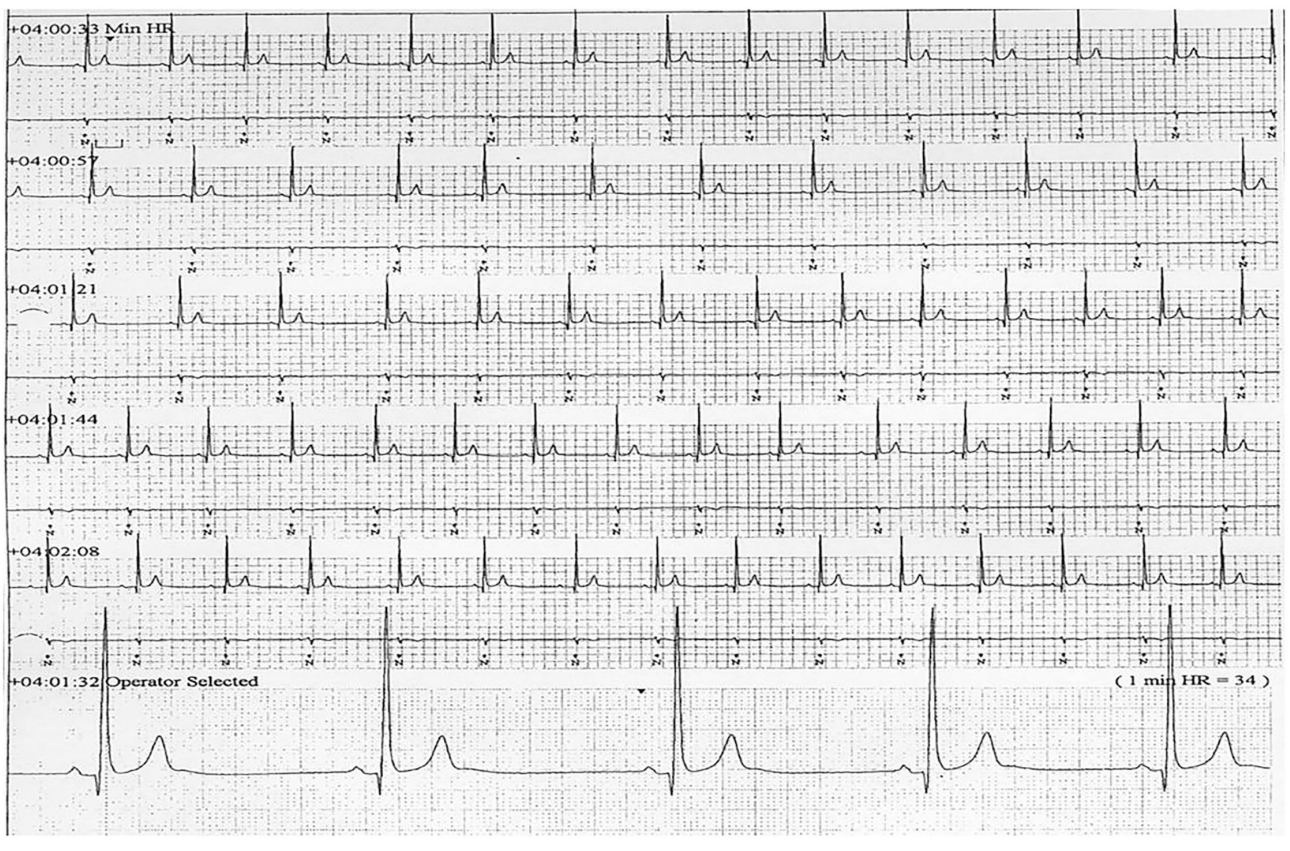

FIGURE 1 | ECG transcript of patient 5, aged (26 years of age) showing sinus bradycardia ($24H_{min} = 34$).

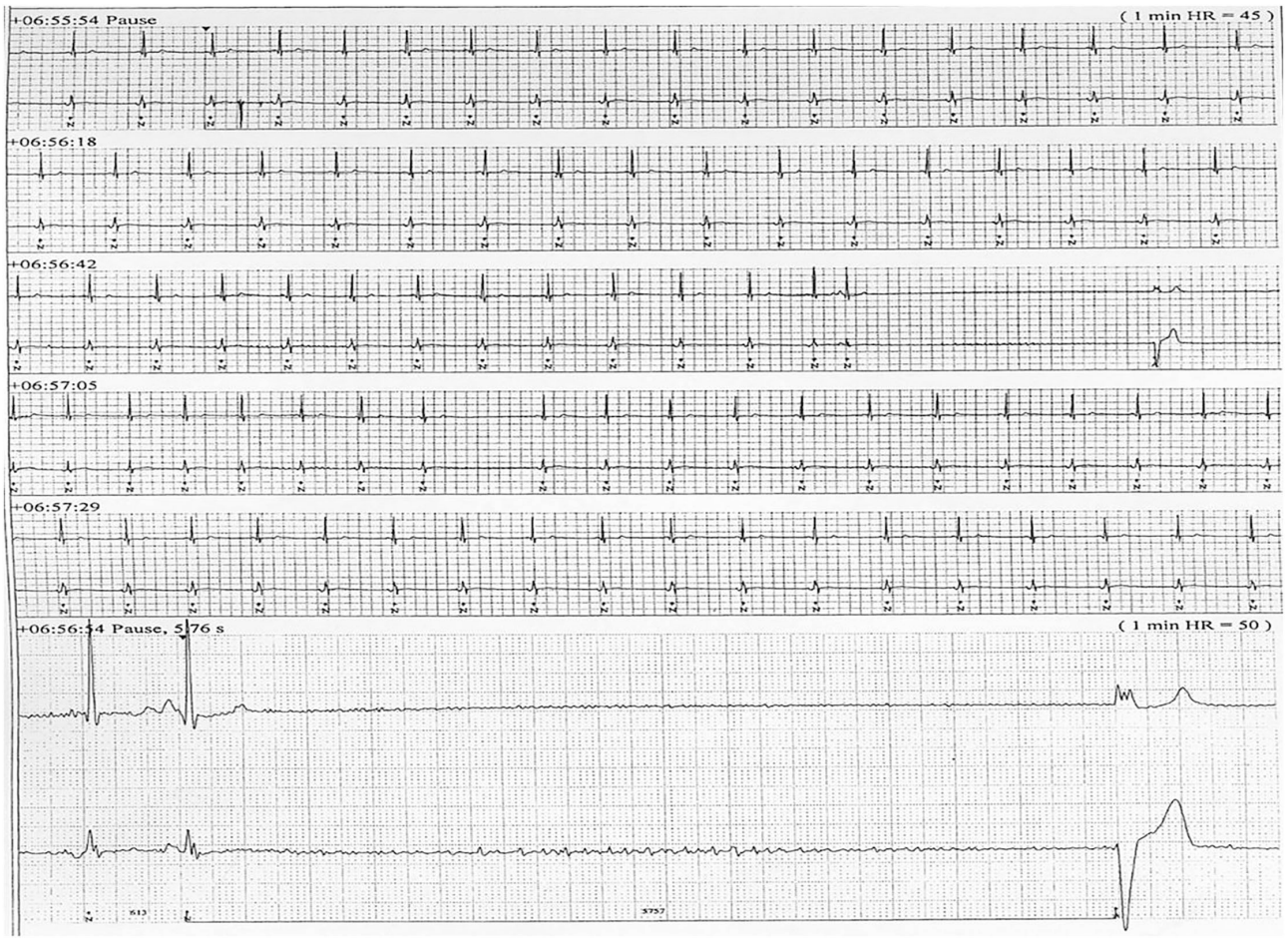

FIGURE 2 | ECG transcript of patient 5, aged (26 years of age) having recurrent sinus arrests, the one shown lasting 5.76 s.

bundle of His, and to a lesser extent inside the cardiomyocytes of CLN3 patients (3, 7). Accordingly, an increasing frequency of conduction disorders including sinus arrest is seen in these patients in their late teens, along with left ventricular hypertrophy in the early 20 s (7).

The experience of pacemaker treatment for CLN3 disease is limited and only case reports have been published. In 2014, two siblings with a specific, not previous described *CLN3* mutation both received a pacemaker due to severe bradycardia and sick sinus syndrome (4). Although they later received an implantable cardiac defibrillator, the cardiac function deteriorated to cardiac failure. In a single patient reported by Dilaveris et al. (5), pacemaker implantation resulted in a more alert patient, and previous reported difficulties with dysphagia and choking during mealtimes disappeared. In the present study, we describe the clinical course of six adult patients in which pacemaker implantation was indicated from a cardiac point of view. Four of them ended up having a pacemaker. Three patients were still alive 9 years later, and in all patients the caregivers reported that they have a better quality of life. Their activity levels were increased, their temperature of the peripheral extremities remained normal, and there had been no shortness of breath and no cardiac related fainting. All three patients now showed ventricular hypertrophy, and their motor and linguistic abilities continue to regress following pacemaker implantation, indicating a further ongoing progression of the underlying CLN disease.

Our follow-up cohort consists exclusively of males, which might indicate a gender difference. It might be related to the small number of participants. However, sex difference in CLN3 disease course has been reported in both a North American and Danish study (11, 12) where females demonstrated earlier loss of independent functions, had lower quality of life, and died approximately 18 months earlier. In addition, females demonstrate cardiac pathology earlier as do males (7), and as we did not recommend pacemaker implantation in patients with a severely affected general health condition, the apparent gender difference might be real.

In our study we did not have an explicit definition of what eventually should lead to pacemaker removal. One could be concerned that having a pacemaker in a progressive and ultimately lethal disease might lead to an unnecessary extended course of the disease at the terminal phase. However, one patient

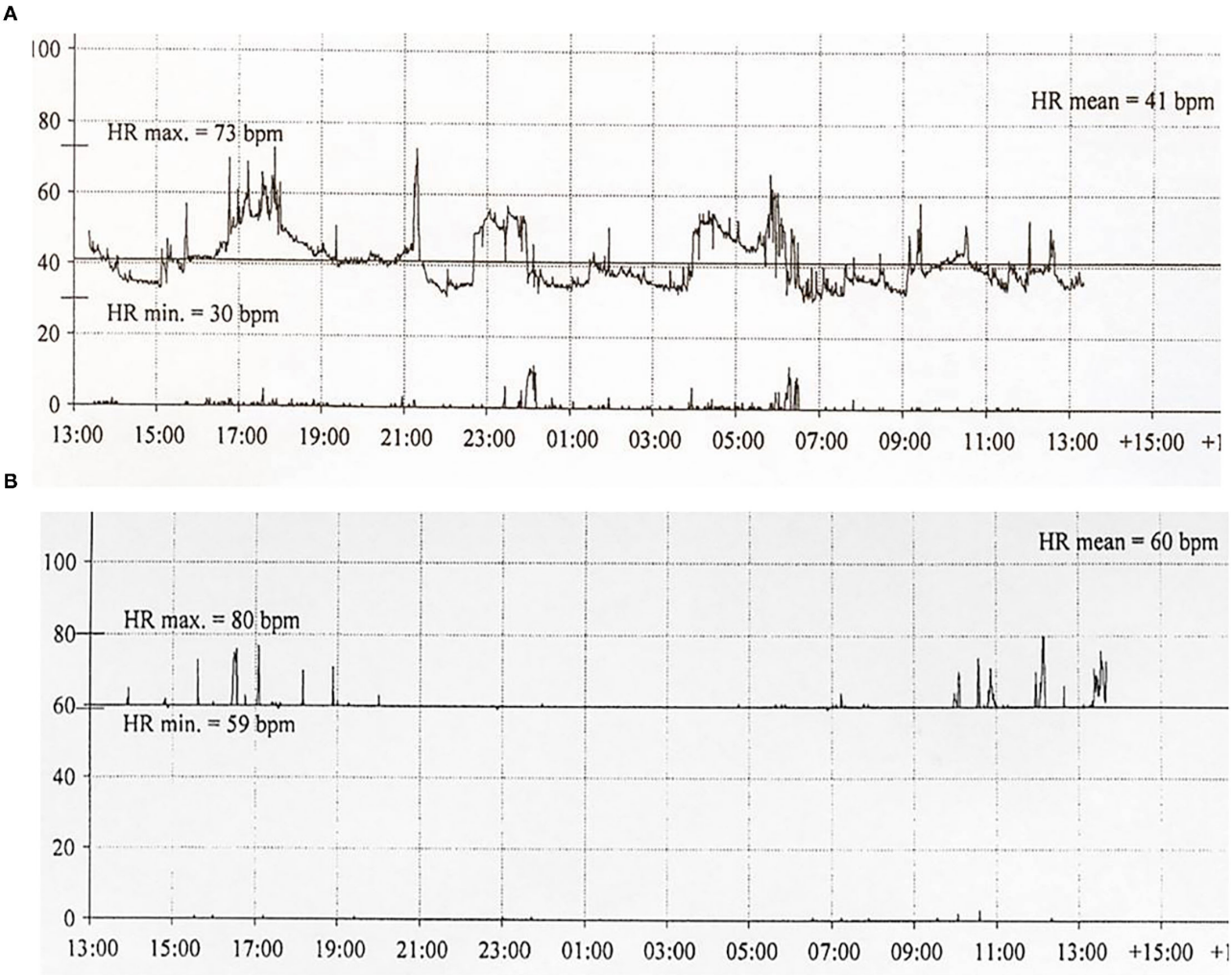

FIGURE 3 | Twenty-four hour ECG transcripts of Patient 4 (21 years of age) before **(A)** and 3 months **(B)** following pacemaker implantation.

died due to pneumonia already 1 year following pacemaker implantation. Thus, having a pacemaker seems not to affect the real terminal phase, but we cannot exclude that pacemaker implantation is a life-prolonging procedure, and we therefore sincerely encourage to a thorough dialog with parents before decision of a potential pacemaker implantation has to be made. In two patients, both with long-lasting sinus arrests, pacemaker implantation was deselected. They both were in the terminal phase, and although it cannot be known with certainty, pacemaker treatment would hardly have extended their lives significantly.

The field of NCL research is currently evolving rapidly and progress in gene therapy may be glimpsed in the horizon (8, 9). Nevertheless, we know from the progress in CLN2 treatment that therapy may only be effective in some organ systems (13). In the close future we face gene therapy or other new treatment regimens in patients with CLN3 disease, but whether these treatments will also be effective outside the central nervous system such as the heart, still remain to be elucidated.

Limitations

The study comprised a small number and although the patients were followed consecutively by only one or two different clinicians during follow-up, the follow-up study has a retrospective design as well. Only CLN3 patients having the common homozygous deletion of exon 7/8 in the CLN3 gene participated, and a phenotypic variability in cardiac symptoms is still to be expected. Additionally, related to our inclusion criteria for pacemaker implantation, we might have a selected a group of CLN3 patients with a relatively benign disease course.

CONCLUSIONS

Our study confirms that severe cardiac conduction disturbances, which principally requires decision regarding pacemaker treatment, is relatively common in patients with CLN3 and leads to symptoms reducing quality of life, including syncope fainting. A pacemaker implantation is a safe procedure in CLN3 patients, and in the present case-series it improved their quality of life

and prevented further episodes of syncope. We cannot exclude that the procedure may be life-prolonging and will emphasize the importance of a thorough information of parents/caregivers when decision of a potential pacemaker implantation has to be made.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Regional Legal Office of the Central Denmark Region, Journal no 1-45-70-72-21, August 25, 2021. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MH and JO have conceived and designed the follow-up protocol and made first draft. JO, MH, and BA conducted the follow-up study. HM performed and evaluated the cardiac examinations. All authors have approved the final manuscript.

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REFERENCES

1. Nita DA, Mole SE, Minassian BA. Neuronal ceroid lipofuscinoses. *Epileptic Disord.* (2016) 18:73–88. doi: 10.1684/epd.2016.0844
2. Mole SE, Cotman SL. Genetics of the neuronal ceroid lipofuscinoses (Batten disease). *Biochimi Biophys Acta.* (2015) 1852:2237–41. doi: 10.1016/j.bbadis.2015.05.011
3. Rietdorf K, Coode EE, Schulz A, Wibbeler E, Bootman MD, Ostergaard JR. Cardiac pathology in neuronal ceroid lipofuscinoses (NCL): more than a mere co-morbidity. *Biochim Biophys Acta.* (2020) 1866:165643. doi: 10.1016/j.bbadis.2019.165643
4. Cortese A, Tucci A, Piccolo G, Galimberti CA, Fratta P, Marchioni E, et al. Novel CLN3 mutation causing autophagic vacuolar myopathy. *Neurology.* (2014) 82:2072–6. doi: 10.1212/WNL.0000000000000490
5. Dilaveris P, Koutagiar I, Aggeli C, Sideris S, Gatzoulis K, Stefanadis C. Severe sinus node dysfunction in a patient with juvenile neuronal ceroid lipofuscinosis. *Int J Cardiol.* (2014) 174:143–6. doi: 10.1016/j.ijcard.2014.03.159
6. Hofman IL, van der Wal AC, Dingemans KP, Becker AE. Cardiac pathology in neuronal ceroid lipofuscinoses–a clinicopathologic correlation in three patients. *Eur J Paediatr Neurol.* (2001) 5(Suppl A):213–7. doi: 10.1053/ejpn.2000.0465
7. Ostergaard JR, Rasmussen TB, Mølgaard H. Cardiac involvement in juvenile neuronal ceroid lipofuscinosis (Batten disease). *Neurology.* (2011) 76:1245–51. doi: 10.1212/WNL.0b013e31821435bd
8. Cooper JD, Mole SE. Future perspectives: what lies ahead for Neuronal Ceroid Lipofuscinosis research? *Biochim Biophys Acta.* (2020) 1866:165681. doi: 10.1016/j.bbadis.2020.165681
9. Mole SE, Anderson G, Band HA, Berkovic SF, Cooper JD, Kleine Holthaus S-M, et al. Clinical challenges and future therapeutic approaches for neuronal ceroid lipofuscinosis. *Lancet Neurol.* (2019) 18:107–16. doi: 10.1016/S1474-4422(18)30368-5
10. Mølgaard H, Sørensen KE, Bjerregaard P. Minimal heart rates and longest pauses in healthy adult subjects on two occasions eight years apart. *Eur Heart J.* (1989) 10:758–64. doi: 10.1093/oxfordjournals.eurheartj.a059561
11. Cialone J, Adams H, Augustine EF, Marshall FJ, Kwon JM, Newhouse N, et al. Females experience a more severe disease course in Batten disease. *J Inherited Metab Dis.* (2012) 35:549–55. doi: 10.1007/s10545-011-9421-6
12. Nielsen AK, Østergaard JR. Do females with juvenile ceroid lipofuscinosis (Batten disease) have a more severe disease course? The Danish experience. *Eur J Paediatr Neurol.* (2013) 17:265–8. doi: 10.1016/j.ejpn.2012.10.011
13. Schulz A, Ajayi T, Specchio N, de Los Reyes E, Gissen P, Ballon D, et al. Study of Intraventricular cerliponase alfa for CLN2 Disease. *N Engl J Med.* (2018) 378:1898–907. doi: 10.1056/NEJMoa1712649

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frontiers in Neurology

REVIEW
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Neuronal Ceroid Lipofuscinosis: The Multifaceted Approach to the Clinical Issues, an Overview

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The main aim of this review is to summarize the current state-of-art in the field of childhood Neuronal Ceroid Lipofuscinosis (NCL), a group of rare neurodegenerative disorders. These are genetic diseases associated with the formation of toxic endo-lysosomal storage. Following a brief historical review of the evolution of NCL definition, a clinically-oriented approach is used describing how the early symptoms and signs affecting motor, visual, cognitive domains, and including seizures, may lead clinicians to a rapid molecular diagnosis, avoiding the long diagnostic odyssey commonly observed. We go on to focus on recent advances in NCL research and summarize contributions to knowledge of the pathogenic mechanisms underlying NCL. We describe the large variety of experimental models which have aided this research, as well as the most recent technological developments which have shed light on the main mechanisms involved in the cellular pathology, such as apoptosis and autophagy. The search for innovative therapies is described. Translation of experimental data into therapeutic approaches is being established for several of the NCLs, and one drug is now commercially available. Lastly, we show the importance of palliative care and symptomatic treatments which are still the main therapeutic interventions.

Keywords: neuronal ceroid lipofuscinosis, NCL clinical features, NCL pathogenetic mechanisms, NCL treatments, NCL review

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INTRODUCTION: HISTORICAL NOTES

The Neuronal Ceroid Lipofuscinoses (NCL) are neurodegenerative disorders, mostly of childhood onset. They form a heterogeneous group of lysosomal storage diseases (LSD) mainly affecting brain and retina (1). They are genetic disorders, and the first description of putative juvenile NCL was of four siblings in Norway with progressive visual loss, cognitive decline, seizures and premature death. This report remained unnoticed until 150 years later (2).

Cases of progressive visual loss with cognitive decline of infantile and/or childhood onset and a fatal outcome were grouped around the turn of the 19th century under the term "amaurotic familial idiocy", coined by the American neurologist Sachs (3), and included the ocular manifestations described by the British ophthalmologist Tay (4). Several familial cases were described from different European countries. The contribution of neuropathology further characterized this group of diseases by describing the topography of brain abnormalities and the selective involvement of the cerebral and cerebellar cortices and of subcortical gray nuclei (5–9). Adult onset cases were reported by Kufs (10). The common appearance of swollen cerebral neurons (as well as retinal

ganglion cells), whose topology was distorted because of the cytoplasmic engulfment with granular materials with similar staining properties was also described (11). Batten (12) and Spielmeyer (8) went on to outline some differences in the features and distribution of storage material between these entities, allowing division of the amaurotic familial idiocies into different clinical entities with different genetic backgrounds (13), specific biochemical properties (14, 15), selected neuropathological features (16), and eventually genetic markers toward the end of the last century. This was the beginnings of the classification and nosography of so-called storage diseases.

NCL are currently grouped under two major eponyms, Batten disease and Kufs disease. Batten disease refers to childhood NCLs, regardless the age of onset, whereas the term Kufs disease is assigned to the two major phenotypes of adult onset NCL [Kufs A and B (17)]. NCL definition relies on pathological criteria: the presence of autofluorescent lipofuscin and the characteristic cytosomes. Zeman and colleagues introduced the term "Neuronal Ceroid Lipofuscinosis" based on the histochemical and ultrastructural features (18). The identification of such markers allowed the NCLs to be further characterized and distinguished from other "amaurotic familial idiocies", such as gangliosidoses (19).

Intracytoplasmic accumulation of autofluorescent material is a pathogenetic hallmark of the NCLs, and it is ascribed to the abnormal storage of ceroid, a pathologically derived material, with similar biochemical properties to lipofuscin, the "aging pigment" (20). The abnormal storage material is embedded within lysosomes, and its ultrastructural features probably reflect the chemical composition of the storage and the related aggregates (16). The biochemical composition of the storage is still only partially defined. A main component (subunit c of the mitochondrial ATP synthase) accumulates in the late infantile variants and in juvenile onset NCL; sphingolipid activating proteins (Saposins A and D) are enriched in two infantile onset forms (21, 22). Interestingly these components are detected in CNS tissue only, and they are not present in peripheral tissues, where the storage can be detected in several cell types ultrastructurally.

The recognition of lysosomal involvement in the NCLs was followed by a huge research effort aiming to disentangle the pathogenetic mechanisms and to shed light on the cellular pathways and processes which are affected (see Section The Research Contribution to Knowledge).

The identification of lipopigment storage ultrastructurally became the main diagnostic tool for NCL, and cerebral biopsies represented the favored diagnostic approach. Evidence that storage material could be detected in readily accessible extra-neural tissues (such as skin, blood lymphocytes, skeletal muscle) led to an important shift from CNS to peripheral biopsies, a safer and more rapid pathway to diagnosis (23). Peripheral neurons, such as intramural ganglionic neurons of rectal mucosa, were also utilized for diagnostic purposes (24, 25). The impaired function of these cells was associated with gastrointestinal problems, including abdominal pain, constipation, and altered bowel motility in NCL patients. The involvement of the enteric nervous system was recently investigated in three NCL mouse models, which showed both impaired enteric functions and histological and ultrastructural findings consistent with neuronal loss and storage accumulation (26).

Based on clinical and pathological criteria, the NCLs were classified according to age of clinical onset and the ultrastructural features of the cytosomes. Their nomenclature was modified over time to include newly recognized variants [for example, infantile and early juvenile] and the adult form (16, 27, 28). Such classification has been valid for about four decades, and it is still useful in a clinical setting (**Table 1**). It has helped to target molecular genetic diagnostic investigations. The identification of cytosomes in the peripheral tissues of unusual cases, where the clinical phenotypes is not entirely consistent with known NCL genetic types, may lead to molecular analysis using NCL panels, sometimes obtaining diagnostic confirmation at molecular level (29). Major steps in NCL history are summarized in **Figure 1**.

An axial classification system was proposed, including seven axes, to obtain information necessary to better categorize each NCL form, according to the specific items which characterize them (30) and represents a useful diagnostic tool for research purposes.

GENERAL CLINICAL ISSUES

Advances in Genetics

NCL are genetic diseases (13). All childhood and most adult NCLs are inherited as autosomal recessive diseases. There is only one dominantly transmitted adult-onset form, Parry disease, associated with mutations in *CLN4*.

A new age in the NCL history started during the last decade of the 20th century. The first human NCL genes, *CLN1* and *CLN3*, were identified in 1995 by positional cloning (31, 32). The identification of the remaining eleven NCL genes occurred within the following decade, making use also of naturally occurring animal disease models. The pathogenicity of identified mutations was proven by different approaches *in vivo* (eg. using knock-out invertebrate and mammalian models) and/or *in vitro*.

The consequences of the genetic advances are multifaceted. From a clinical perspective it allowed a new gene-based classification and NCL nomenclature (33), provided a powerful tool for diagnosis, and helped clinicians recognize both phenotypic variability and heterogeneity within most NCL genetic disease types (34). The classical phenotypes are generally associated with the most common mutations in each gene, whereas variant forms may arise from "private" mutations. Phenotypic variability can be observed even within some families, which, for example, may lead to differences in survival among siblings. Advances in NCL genetics also gave the opportunity to re-evaluate NCL epidemiology and to recognize the worldwide distribution of this group of diseases (see below Section Epidemiology and Registries). The identification of the NCL genes and their related products helped to begin to identify altered cellular processes and patho-mechanisms leading to cell death and progressive neurodegeneration which characterizes all NCLs. The cross-talk between different cell compartments (eg lysosomes and endoplasmic reticulum, lysosomes and mitochondria) has been described in some NCL forms (35–38)

TABLE 1 | NCL of childhood onset: clinical classification and major diagnostic procedure.

Clinical form	Age of onset	Disease	Gene	Diagnosis	Major symptoms at onset
Congenital	Birth	CLN10	*CLN10/CTSD*	NGS Enzymatic assay	Microcephaly, dysmorphic features, seizures, hyperkinetic movements;
Infantile	6–18 months	CLN1	*CLN1/PPT1*	NGS enzymatic assay	Decreased head growth, neuro-developmental regression, seizures;
		CLN10	*CLN10/CTSD*	NGS enzymatic assay	Decreased head growth, neuro-developmental regression;
		CLN14	*CLN14/KCDT7*	NGS	Decreased head growth, seizures (myoclonus);
Late infantile					
Classical	2–4 yrs	CLN2	*TPP1*	NGS enzymatic assay	Seizures, ataxia, visual loss, delayed language development;
Variant	2–5 yrs	CLN1	*CLN1/PPT1*	NGS enzymatic assay	Seizures, neuro-developmental regression, behavioral disturbances;
		CLN5	*CLN5*	NGS	Impaired learning and cognition;
		CLN6	*CLN6*	NGS	Seizures, ataxia, delayed language development;
		CLN7	*CLN7/MFSD8*	NGS	Seizures, visual loss, motor and cognitive regression;
		CLN8	*CLN8*	NGS	Seizures, visual loss, motor and cognitive regression;
Juvenile					
Classical	3–5 yrs	CLN3	*CLN3*	NGS	Visual loss, behavioral problems, cognitive decline
	5–7 yrs	CLN5	*CLN5*	NGS	Motor and cognitive regression, behavioral problems;
	5–7 yrs	CLN1	*CLN1/PPT1*	NGS enzymatic assay	Visual loss, cognitive decline;
Late	8–12 yrs	CLN6 CLN10	*CLN6* *CLN10/CTSD*	NGS NGS enzymatic assay	Myoclonic seizures, cognitive decline; ataxia, cognitive decline, visual loss;
	13–16 yrs	CLN12	*ATP13A2*	NGS	Rigidity, hypokinesia

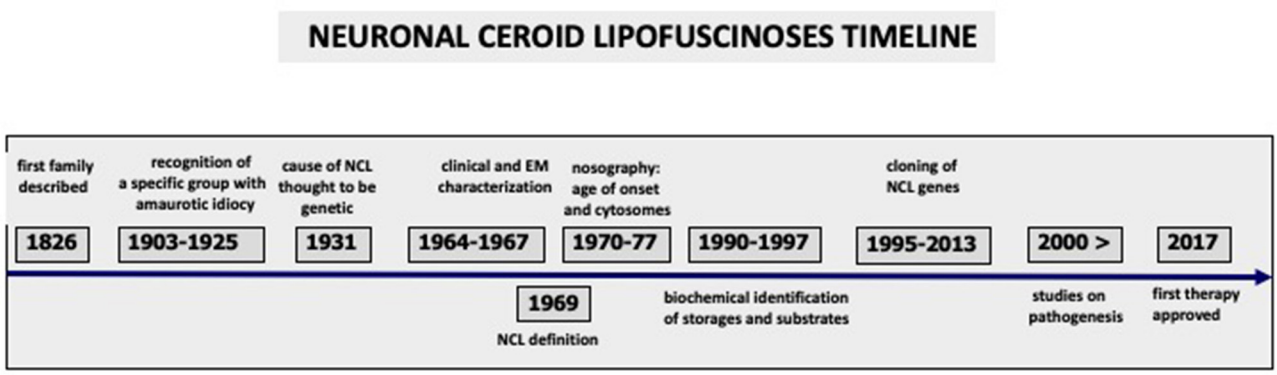

FIGURE 1 | Major steps and achievements during nearly two centuries of Neuronal Ceroid Lipofuscinoses history are outlined, starting from the earliest clinical description to the present research which led to the first FDA/EMA approved treatment.

and has helped to shed light on some clinical features which cannot be ascribed to the mutated NCL protein only. Animal and cellular models have been generated to expand our knowledge of the pathology, and so the first disease modifying and therapeutic agents are now becoming available for clinical trials (39). The first such agent was approved for clinical use in 2017 following a pivotal phase I/II clinical trial.

As shown in **Table 2**, allelic adult-onset variants are observed in several childhood forms (40). In most however, the adult phenotype shows clinical features which are consistent with the classical childhood form and are differentiated only by the age of onset. The well-defined adult onset NCLs are still named by classical eponyms [Kufs A, Kufs B and Parry diseases]. They are associated with pathogenic mutations of *CLN6* [Kufs A disease, (41)] and with three other genes (*CLN4, CLN11, CLN13*) whose mutations give rise exclusively to adult-onset phenotypes (42–45).

The identification of childhood NCL genes is also contributing to the identification of potential genetic signatures for neurodegeneration in adulthood. A number of NCL genes

TABLE 2 | Characterization of adult onset NCL.

Form	MIM	Inheritance	Chromosome	Gene	Gene product	Onset (decade)	Symptoms at onset
KD-A/CLN6	#204300	AR	15q23	*CLN6*	CLN6: transmembrane protein (ER)	2nd-5th	Seizures, action myoclonus, ataxia, cognitive decline
KD-B/CLN13	#615362	AR	11q13.2	*CLN13/CTSF*	CTSF (soluble protein): lysosomal enzyme	2nd-7th	Seizures, myoclonus, cerebellar tremor, cognitive decline, depression, anxiety
Parry/CLN4	#162350	AD	20q13.33	*CLN4/DNAJC5*	CSPα (soluble protein): cytosol (vesicular membrane)	3rd-5th	Seizures, action myoclonus, visual failure
CLN11	#614706	AR	17q21.31	*CLN11/GRN*	Progranulin (soluble protein)	3rd	Seizures, visual failure
CLN1	#256730	AR	1p34.2	*CLN1/PPT1*	PPT1 (soluble protein): enzyme (lysosome and extra-lysosomal compartments)	3rd-4th	Cognitive decline, psychiatric symptoms
CLN5	#256731	AR	13q22.3	*CLN5*	CLN5: lysosomal membrane (other cell compartments)	6th	Unsteady gait

KD, Kufs disease; ER, endoplasmic reticulum.

share mutations or are allelic with mutations contributing to common adulthood neurodegenerative disease, such as Alzheimer's Disease, Fronto-Temporal Dementia, Parkinson's Disease (46–48). Moreover, lysosomal dysfunction and dysregulated autophagy which are observed in CLN3 and CLN6 disease (49, 50) are also seen in most forms of late-adulthood neurodegeneration (51, 52).

Advances in NCL genetics were accompanied by new opportunities to establish diagnosis at a biochemical level; enzymatic assays became available for four lysosomal enzymes: CTSD, CTSF, PPT1, and TPP1 (53–55). The gold standard for diagnosis is to require genetic confirmation by detecting both disease causing alleles. Therefore, when detection of the common disease causing mutations is not confirmed in the presence of a biochemical impairment, the diagnostic search for changes in cryptic gene regions (introns, untranslated regions, etc.) is extended. The requirement to establish a molecular diagnosis is of a primary importance for families helping to inform prognostic counseling, for antenatal diagnosis and, more recently, to judge suitability for "innovative" treatments where they are available. The recent availability of NGS technology has changed the clinical approach to NCL diagnosis, which relies on a straightforward molecular approach, leaving the neuropathological and ultrastructural investigations to cases which remain unsettled, even after molecular analysis (**Figure 2**).

Epidemiology and Registries

NCL are rare diseases which are distributed world-wide. The higher prevalence of selected NCL forms in restricted geographical areas has some historical relevance and may also reflect the early progress in molecular diagnosis in some countries (31, 56, 57). Epidemiological data indicate an incidence of 1–3/100.000 and a prevalence of about 2–4/1.000.000 (58–62). These figures refer to Western countries where access to molecular diagnosis has become standard of care over the last 10–15 years. More detailed epidemiological study is necessary to improve awareness of these diseases, the efficacy of genetic counseling, to plan appropriate services, and to facilitate access to "new" treatments. In Western countries CLN3 disease (juvenile NCL) is the most common form, followed by the classical late infantile form, CLN2 disease which is more frequent in Southern Europe and the Mediterranean region. Ultrarare childhood NCL are CLN10, CLN12, CLN14. A major contribution to the dissemination of knowledge comes from disease registries and databases. The independent International NCL Database (led by Dr A Schulz in Hamburg, Germany; Clinical trials identifier NCT04613089, an extension of former NCL DEM-CHILD patient database) is collecting static and dynamic clinical data of NCL patients from 19 countries from Europe, North and South America and Asia. The UCL based NCL Mutation Database (established in 1998 by prof S.E. Mole; http://ucl.ac.uk/ncl-disease/) lists all published and reported NCL patients as well as mutations.

The Current Nosography

The impressive amount of data collected over the last two decades has allowed the NCL community to generate a new nomenclature of NCL based on genetic variations (33), followed by comprehensive reviews which have described the clinical features, the cell localization and functions of the mutated gene products, as well as the historical markers which characterize NCL among other progressive neurological diseases with endo-lysosomal storage. For a systematic review of the clinical and diagnostic features of each NCL disease (including the phenotypic heterogeneity), readers are referred to recent publications (1, 63–69). A comprehensive classification of NCL is given in **Table 3**.

SELECTED CLINICAL FEATURES

As mentioned in the introduction, the NCLs were grouped as amaurotic idiocies around the end of the 19th century, along with other diseases which were recognized as different disorders later. "Idiocy", progressive blindness, and seizures, remain the cardinal symptoms of the NCLs. The NCLs are the most common neurodegenerative diseases in childhood, and they are one of

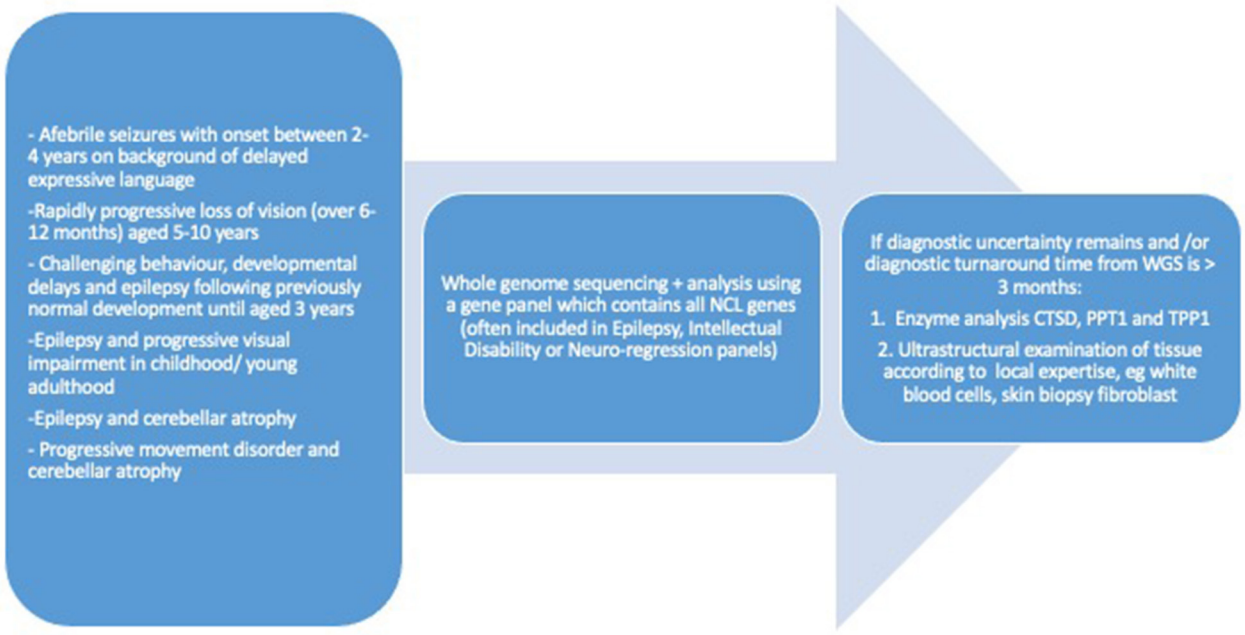

FIGURE 2 | Diagnostic work up for neuronal ceroid lipofuscinosis of childhood onset.

the main causes of childhood dementia worldwide. They are progressive with severe physical decline, and an early death. Infantile and late infantile onset NCLs show the most rapid rate of disease progression. Most patients are bedridden by their second decade, and death occurs during the late second-early third decade. A less rapid course is seen in patients affected with CLN3 disease. Staging systems based on severity scores of selected clinical manifestations have been developed to monitor the disease evolution quantitatively. These tools are helpful in describing the natural history of a disease and are of particular relevance when the efficacy of "new" treatments is tested (70–72).

Cognitive decline, ataxia, amaurosis, and early seizures are the clinical manifestations at onset in most cases. They become evident sequentially over a short time frame (6–24 months) in infantile and late-infantile NCLs; spasticity follows leading to loss of motor function, dependence on carers for all activities of daily living within 5–8 years of symptom onset.

In this review we will discuss the clinical symptoms which characterize the NCLs in more detail, and are shared between the forms, focusing on their temporal evolution at onset and during the early stages of the disease course. Seizures, motor deterioration (including ataxia, movement disorders, spasticity), cognitive decline, progressive blindness, and behavioral problems are the major clinical features of infancy and childhood onset NCLs. The identification of the clinical markers and their relationship with patients' age at onset may help to drive diagnostic procedures toward a specific NCL type, which can be confirmed molecularly. Such an approach reflects the clinical experience of the authors in the field of childhood neurodegeneration.

Epilepsy and Seizures

Epilepsy is common to almost all NCL forms. Several seizure types are seen in the NCLs. The severity of epilepsy (in terms of age of onset, seizure semiology, seizure burden and response to anti-seizure medications) is not shared uniformly. Seizures tend to start earlier and semiology is more varied in infantile and late-infantile onset NCLs. With time seizures tend to become less frequent, except for myoclonus (either spontaneous or evoked) which remains the only paroxysmal manifestation in the late stages of the disease. The background EEG progressively deteriorates leading to a nearly isoelectric pattern, as an expression of progressive cortical atrophy and degeneration of the "generators" of cortical electric activity.

Frequent perinatal *convulsions* (or even status epilepticus) associated with severe cortical and cerebellar atrophy are observed in the ultrarare congenital NCL, which leads to death within the first weeks of life and are usually associated with mutations in *CTSD* (73).

Generalized seizures, *including myoclonus*, starting 2 to 6 months after the earliest clinical manifestations, characterize infantile onset NCL (infantile CLN1 and CLN10 diseases). Before the onset of clinical seizures, EEG abnormalities can be detected, which evolve over time into a characteristic pattern, the "vanishing EEG", observed also in the late infantile variant of CLN1 disease (74–76).

Generalized motor seizures, absences, and myoclonus (including negative myoclonus) are the main seizure types observed in classical CLN2 disease. They are also seen in CLN6, CLN7 and CLN8 late infantile variants. In these forms seizures can be present at disease onset, or appear within

TABLE 3 | NCL: genetic, biochemical and ultrastructural features of each form.

NCL disease	MIM	Gene locus	Mutated gene	Gene product	Cellular compartment	Function	Ultrastructural features
CLN1	#256730	1p34.2	*CLN1/PPT1*	PPT1 (soluble protein)	Lysosomal matrix (acidic ph); extralysosomal vesicles (not acidic pH);	Enzyme (s-acylated protein thioesterase)	GROD
CLN2	#204500	11p15.5	*CLN2/TPP1*	TPP1 (soluble protein)	Lysosomal matrix	Enzyme (serine protease)	CVB (FPP)
CLN3	#204200	16p11.2	*CLN3*	CLN3 (membrane protein)	TMD (late endosomes, lysosomes, presynatic vesicles, axons)	Multiple functions in several cell processes	FPP, vacuoles
CLN4	#162350	20q13.33	*CLN4/DNAJC5*	DNAJC5 (soluble protein)	Cytosol (vesicular membrane)	Co-chaperone (endo/exocytosis)	GROD
CLN5	#256731	13q22.3	*CLN5*	CLN5 (soluble protein)	Lysosomal membrane and matrix, ER, neurites	Endocellular trafficking between cell compartments	Mixed pattern (CVB, FPP, RLP)
CLN6	#601780	15q21-23	*CLN6*	CLN6 (membrane protein)	TMD (ER)	Mediates ER exit of new lysosome enzymes (?)	FPP
CLN7	#610951	4q28.2	*CLN7/MFSD8*	MFSD8 (membrane protein)	TMD (lysosomes, late endosomes; photoreceptor vesicles)	Transporter?	Mixed pattern (CVB, FPP, RLP)
CLN8	#610003 and #600143	8p23.3	*CLN8*	CLN8 (membrane protein)	TMD (ER/ERGIC) (cargo receptor)	Lysosome biogenesis regulator	Mixed pattern (CVB, FPP, RLP)
CLN10	#610127	11p15.4	*CLN10/CTSD*	Cathepsin D (soluble protein)	Lysosomal matrix	Enzyme (aspartyl-endopeptidase)	GROD
CLN11	#614706	17q21.31	*CLN11/GRN*	Progranulin (soluble protein)	Extracellular matrix	Not known	FPP
CLN12		1p36.13	*CLN12/ATP13A2*	CLN12 (membrane protein)	TMD (lysosomes)	Enzyme (P-type ATPase)	FPP
CLN13	#615362	11q13.2	*CLN13/CTSF*	Cathepsin F (soluble protein)	Lysosomal matrix	Enzyme (cysteine protease)	FPP
CLN14	#611725	7q11.21	*CLN14/KCTD7*	Potassium Channel Tetramerization Domain-containing Protein 7 (soluble protein)	TMD (plasma membrane)	Voltage-gated potassium channel complex	Mixed pattern (CVB, FPP, RLP)

TMD, transmembrane domain; ER, endoplasmic reticulum; ER/ERGIC, endoplasmic reticulum/endoplasmic reticulum-Golgi intermediate compartment; GROD, granular osmiophilic deposits; CVB, curvilinear bodies; FPP, finger print profiles; RLP, rectilinear profiles.

1–2 years. A characteristic EEG feature is the paroxysmal spike-wave response, which is evoked by low-frequency (1–3 Hz) intermittent photic stimulation which is observed in CLN2 and CLN6 (late infantile variant) patients during the early stages of the disease (77, 78).

A prevalent *myoclonic epilepsy with progressive features* characterizes the late juvenile (teen age) onset *CLN6 disease* and its allelic adult Kufs A disease. Both forms present with progressive myoclonic epilepsy at onset gradually increasing in frequency and intensity, and less frequent bilateral tonic-clonic seizures. A low-frequency (1–5 Hz) photo-paroxysmal response is present in the vast majority of cases (79). The disease has a slowly progressive course, and patients become wheel-chair bound within few years due to the relentless myoclonus and progressive ataxia (80). Progressive myoclonus epilepsy associated with rapidly progressive dementia is also the clinical hallmark of the dominantly inherited NCL (Parry disease) (42).

Bilateral tonic-clonic seizures (even with focal onset) and *absences* are the most frequent seizure type in CLN3 disease, rarely occurring as presenting symptoms; myoclonus is less frequent than in other NCLs (81, 82).

Major Neurological Symptoms

Motor Deterioration

Ataxia, weakness, loss of acquired motor abilities and spasticity are the most common motor symptoms in NCL patients. The consequence is the loss of motor autonomy and increasing dependence on caregivers. Signs and symptoms of motor impairment become evident in children who had normal motor development previously.

Ataxia is the most frequent clinical symptom of CLN2, CLN7, CLN8 disease and of the rarer late-infantile variant of CLN1 disease whose age of onset is between 2 and 5 years. It is also evident in CLN6 disease but onset is 1–2 years later. Ataxia in the NCLs is secondary to spino-cerebellar involvement. Purkinje cells and neurons of the deep cerebellar nuclei are severely affected and undergo early death. Cerebellar atrophy is an early neuro-radiological sign, preceding the enlargement of cortical sulci and of the lateral ventricles. Sometimes the

term "ataxia" is incorrect, and the transient spells of motor instability which a child shows should be considered as clinical expression of "negative myoclonus", which is commonly present in these forms.

Motor weakness affecting limbs, eye muscles (strabismus) and deglutitory muscles (dysphagia) is likely to occur because of impaired cortico-descending connectivity due to the involvement of motor cortex and the neurons of motor nuclei. The progressive degeneration of motor pathways is followed by *spasticity* which represents further disease progression. Changes in gait phenotypes in different forms of Batten disease has been recently considered as a "marker of disease progression" (83).

The progressive involvement of cortical neurons also leads to a progressive *loss of motor skills, motor initiative, motor planning and strategies*, which represent the "cognitive foundation" of motor function. This same process also leads to dementia. Declining motor function is observed in all NCL forms, leading to loss of independent ambulation, subsequent loss of all voluntary movement and posture control, the rate of decline dependent on the NCL type. Children with the infantile and late infantile NCL types become wheel-chair bound at 6–12 years of age (according to the severity of the form) before becoming "bed-ridden" (early teens in late infantile forms, early-mid second decade in CLN3 patients).

Extrapyramidal symptoms, such as tremor and rigidity, are known to occur in NCL, being particularly relevant in CLN3 disease. They are responsive to available treatments, such as anti-cholinergic drugs, Dopamine. Dystonia and chorea can be recognized at different stages of NCL disease course (84, 85). The reasons why basal ganglia structures and/or neuronal connections underlying these clinical manifestations are relatively spared in NCLs is a matter which requires further investigation.

Cognitive Decline and Impaired Language Development

Cognitive decline is hallmark of the NCLs. In younger children the loss of cognitive abilities related to school-learning occurs rapidly, whereas the relatively slow pace of disease evolution observed in "juvenile" patients allows them often to attend mainstream school whilst continuing to learn new facts and skills into their teenage years. From this perspective two major issues should be considered, which further differentiate early onset NCL from juvenile forms.

Cognitive decline occurs as a two-phase problem. Early in the disease, children's developmental trajectory slows, they acquire new learning more slowly than their peers but often remain within the normal range for some time before professionals become concerned. They go on to plateau and then begin to lose the cognitive competences they have acquired during the earliest months and/or years of their life. This is often the time at which diagnostic investigations are triggered. In CLN2 disease however many children show a delayed acquisition of cognitive skills at a very early stage and their profile is characteristically uneven with delays more evident in the domain of expressive language compared with motor skills. This has been reported in recent years and is of huge importance for driving early diagnosis. Nickel et al. (86) reported that about 40% of a cohort of CLN2 patients presented early with delayed language acquisition. It raises the potential to consider language delay as an early warning sign, leading to careful clinical examination of such children, in order to detect any additional concerning features which would justify a deeper diagnostic workup including genetic screening. The atypical early developmental profiles of affected children may also give some clues about the molecular basis of language development, and the role that cellular pathways involved in the development of this complex function may play. There are no studies available for NCL late infantile variants, but it is tempting to hypothesize similar findings in some NCL types at least, e.g. CLN7 and CLN8 disease. Delayed language development in CLN2 disease children does not seem to be related to any specific CLN2 genotype.

Different clinical issues are related to the decline of cognition in CLN3 disease, which can be considered as the prototype of amaurotic idiocy. The rate of disease progression is commonly slow and children can attend school, with support for the intellectual, visual and sometimes behavioral difficulties. There is no evidence to date of an uneven developmental profile early in life and before the onset of visual impairment. The scores of the neuropsychological function tend to diverge from typically-developing peers, reflecting the failure to achieve the expected achievements, but a marked drop in their cognitive abilities occurs during mid-adolescence, at around the same time as rapid neurological decline (87).

Behavioral Problems

Behavioral problems at onset characterize CLN3 and CLN5 diseases, and to a less extent atypical CLN2 disease, three NCLs where first symptoms occur in school age children. There are well described and differentiated disease natural history, and which are caused by mutations in three different nuclear genes whose products have different functions and are located in different cell compartments.

CLN3 disease is the most prevalent NCL in Northern Europe and USA. It has a juvenile (school age) onset with *visual impairment and behavioral problems*, followed by cognitive decline. Motor impairment and epilepsy occur later. Behavioral problems include anxiety, depressed mood, bursts of aggressive behavior and psychotic manifestations. These symptoms tend to remain stable or even worsen during the early years after disease onset, and then become less significant as the disease advances and functional and cognitive abilities are lost. Behavioral problems represent a major challenge to the quality of life for those patients (and their families and carers) where a slow disease course and longer survival is expected (87, 88), and seems also to be unrelated to the genotype (89). The disease evolution is much slower than in other forms, and death may occur in the fourth or even sixth decade, unless cardiac involvement results in premature death (see below). There is a relevant phenotypic homogeneity (possibly related to a common mutation which is observed in the vast majority of cases). Some gender differences have been reported, the female patients presenting a more severe clinical course (90).

Behavioral manifestations and impaired language are the early clinical manifestations of a late infantile "variant", of pre-school age onset, CLN5 disease. Seizures and visual impairment occur 3–4 years after disease onset, followed by progressive motor impairment with spasticity with loss of ambulation by 11–12 years of age. The predicted survival is about 15–20 years after disease onset. In this disease there is some evidence that phenotypic variation (as far as the rapidity of neurological decline and survival) is related to the severity of mutations (91, 92).

*Behavioral disorders (*along with *seizures and, language abnormalities*) at onset were also reported in a large South American cohort of children with *atypical CLN2 disease* (93).

Blindness

Progressive visual impairment is one of the classical symptoms of NCL of childhood onset. It affects all forms, and in the large majority of cases it occurs as one of the early clinical signs. Retinal structures, visual pathways and visual cortices are affected. Both ganglionic neurons and receptor cells (cones and rods) are involved. Impaired retinal response is visualized by ERG. Optical Computerized Tomography allows monitoring of the progressive retinal degeneration. Most published evidence relates to CLN3 and CLN2 diseases (94, 95), and to less extent to CLN1 disease (96). A spectrum of retinal disturbances was described in CLN7 disease and related to the severity of mutations (97). Whether maculopathy precedes cognitive impairment in CLN3 disease, or if both visual impairment and mild cognitive failure occur at around the same time (98) is still the subject of discussion. Biallelic variants of the MFSD8 gene may be associated with isolated juvenile maculopathy, evolving into a slowly progressive encephalopathy with protracted course (99). Conversely, late visual impairment, or even long lasting preservation of visual function can occur in CLN5 and CLN6 diseases (92, 100). Notably, in the allelic Kufs A disease the retina is not affected and vision is normal. Recently, patients affected with isolated retinal degeneration (including retinitis pigmentosa) have been identified harboring biallelic CLN3 and CLN10 pathogenic variants (101, 102).

Therapeutic trials are in progress aiming to prevent retinal degeneration in animal models using intravitreal gene or enzyme therapy (103, 104). These approaches represent powerful therapeutic systems suitable for translation into NCL patients. A major question arises regarding the contribution of central nervous system pathology to functional vision, which is unlikely to be corrected by a retinal only approach.

Sleep Disturbances

Sleep disturbances are common in all NCL types. There are few studies on this issue (105–107) but the importance of sleep management has been outlined in two recent consensus papers (108, 109). Poor sleep quality and sleep pattern disturbance will impact adversely on the quality of life of affected children and their families directly and indirectly through worsening of seizure control and behavior. Supportive measures (such as respite care) and behavioral strategies (such as sleep hygiene) are necessary given the lack of consistent benefit from commonly used medical interventions, such as Melatonin (110). Anti-seizure medications, often prescribed, may help falling asleep, but side effects (e.g. morning drowsiness) impair the restorative function of sleep.

Atypical Cases

It has been known for several years that phenotypic heterogeneity is a feature of genetic diseases (including NCL), and some patients were considered as atypical cases. The origin of clinical variation is commonly ascribed to the genetic background of patients as well to the severity of the mutations. Recently polymorphisms or mutations of unrelated genes have been considered as modifiers of gene expression, and the interactions between mutated genes and modifiers may lead to clinical variations and to the observed phenotypic heterogeneity. In addition non-genetic confounding factors may also affect clinical phenotype.

An "atypical case" has been described in a child affected with a congenital form of CLN5 disease (which usually presents with a late infantile onset, with relatively slow disease progression after onset): he was a compound heterozygote in *CLN5* and was also carrying an incompletely penetrant variant in *POLG1*, a nuclear gene coding for mitochondrial polymerase (111). No evidence was provided of how the co-inherited *POLG1* variant may have enhanced the putative pathogenetic effect of the *CLN5* mutations. However, the interactions between lysosomal and mitochondrial compartments may be implicated, further affecting the physiology and viability of cells hampered by the presence of pathogenic *CLN5* mutations. Another atypical case is related to *CTSD*, commonly associated with congenital or late infantile NCL (CLN10). In a patient with juvenile ataxia associated pigmentary retinopathy and cognitive decline, the evidence of granular osmiophilic deposits (an ultrastructural marker of CLN1, CLN4 and CLN10 disease) in angular atrophic fibers following a muscle biopsy led to the final diagnosis (29). Unusual phenotypes associated with mutations in *CLN2*/*TPP1* with residual leukocyte enzymatic activity raise the issue of the phenotypic heterogeneity, regardless the severity of mutations. A prevailing spino-cerebellar involvement was detected in an adult lady who was suffering from ataxia since adolescence. She was carrying a missense and a splice site variant in *CLN2*/*TPP1* (112). A juvenile onset, progressive and protracted form of cognitive decline with myoclonus, dystonia, bradykinesia and ataxia was reported in three siblings and associated with compound heterozygosity in *CLN2*/*TPP1*, leading to stop codon formation and an aminoacid substitution (113).

It is evident from these "atypical cases", that the detection of a mutated gene represents the starting point to explore the complex mechanisms which ultimately lead to the phenotypic expression, taking into account the unique genetic background which each of us has inherited. Improving our knowledge in this field of "personalized medicine" will help to prevent unexpected and potentially serious adverse events, which might arise when using conventional and novel replacement therapies.

Cardiac Involvement

NCL are considered as LSD. However, they do not show the characteristic multi-organ involvement typical of many LSDs (114), and they predominantly affect the central nervous system. Progressive cardiac involvement is observed in some patients affected with CLN3 disease with repolarization disturbances, ventricular hypertrophy, and sinus node dysfunction (115). Cardiac pace-maker implantation has been used in a small number of patients to improve symptoms of syncopy and extreme bradycardia but is not in widespread use. Twenty four hour ECG recordings have been recommended for annual surveillance in young people age 16–18 years and older. Degenerative changes of the myocardial wall along the deposition of lipopigment and cytosomes can be detected histologically (116).

Heart involvement has also been reported in CLN2 disease [progressive conduction defect; (117)] and hypertrophic cardiomyopathy was described in patients with CLN10 disease (29, 76).

Care and Survival

Reports on life expectancy and mortality in NCL are scarce (118). This issue is assuming particular relevance in natural history studies when assessing the efficacy of "orphan drugs".

The ultra-rare congenital form of NCL has the most dramatic course, the very few reported cases having died within two weeks of birth (119). Ongoing studies confirm that infantile-onset NCL (due to mutated CLN1 and CLN10 genes) and classical CLN2 disease have the next most rapidly severe course and shortest survival. Variability within each form does not allow accurate predictions of life expectancy in individual patients According to the Italian NCL database CLNet, death in the majority of CLN2 patients has been around nine years of age, but prolonged survival is reported in a number of patients affected with the classical form, up to the early third decade, regardless the predicted severity of the mutations they were carrying. Intra-familial variability is also reported. Little is known for the remaining NCLs with infantile and late infantile onset. As mentioned before, the longest survival (up to 4th-6th decade) is observed in CLN3 patients.

Over the last two decades overall longer survival has been observed in patients of many NCL forms, regardless the severity of mutations. Such findings may be ascribed in large part to the improved availability of supportive tools for home care which guarantee appropriate feeding (e.g. percutaneous gastrostomy), respiratory support, as well as the improved quality of skin and general care (e.g. prevention of bed sores), the availability of new anti-epileptic drugs, and the overall improved awareness of caregivers to provide care in order to enhance the length and quality of life of those affected (120, 121).

THE RESEARCH CONTRIBUTION TO KNOWLEDGE

The NCLs are progressive neurodegenerative diseases associated with endolysosomal storage. No cure is available, and major efforts aim to provide the best care to patients. Recently, disease modifying agents have become available or are under investigation for some NCLs (122), by replacing a missing enzyme, the mutated gene, or by reducing the substrate involved in abnormal storage formation. Such approaches reflect the ongoing lack of understanding of the basic disease mechanisms specific to each form, as well as of the shared pathways which characterize the whole group of NCL diseases, despite extensive research efforts.

Experimental paradigms and innovative methodologies have provided major breakthroughs over the last two decades on the knowledge of pathological mechanisms underlying the NCLs (123). In this paragraph we summarize the most relevant data resulting from the current research under different experimental settings, which may contribute to better understanding the rationale of the research toward appropriate strategies to generate new, safe and effective treatments.

Recent studies of outcome on a relatively large population of CLN2 patients are revealing a meaningful slowing down of the rate of disease progression, following the availability of the first "new treatment" (recombinant pTPP1 delivered intrathecally into the ventricular system). Great expectations have risen from the recent announcements of forthcoming treatments whose aim is to both increase the survival and ameliorate the quality of life of the affected children.

Experimental Models

Naturally Occurring Animal Models

Several naturally occurring animal models of NCLs have been discovered and investigated, including non-human primates, ovines, felines, dogs and rodents. The use of relatively large mammals, with convoluted gyral brains represents a powerful tool to examine disease evolution over time (from the early stages until death) and monitor brain pathology using *in vivo* techniques (head imaging, neurophysiology etc) which are currently in clinical use (124–126). In addition, multiple techniques can be utilized to investigate post-mortem brains (and eye) from different perspectives. Along with classical descriptive neuropathology, methodologies are now available which can help to disentangle the complex network of interacting events accompanying the disruptive effects of the intra-neuronal storage in complex mammalian brains. They include the use of immuno-histochemical probes, which allow the detection of selected neuronal and glial populations, to evaluate the distribution and topography of the storage, to check the expression of markers related to pathological processes (such as autophagy), and more recently omics techniques (see below) which allow exploration of expression at the metabolic, protein or even nucleic acid levels of selected biochemical pathways and/or genetic markers (127).

Moreover, these animals are essential in the evaluation of "new" treatments, testing safety, efficacy and putative effects, before progressing to human clinical trials [(39, 128); see Section Pathology and Pathogenetic Mechanisms]. In **Supplementary Table 1** some examples of selected animal models contributing to our understanding of disease mechanisms (and treatment) related to specific human NCL forms are listed.

Engineered Animal Models

Most of the present knowledge of the patho-mechanisms of NCL comes from experimental studies using mouse models which were modified in syntenic NCL genes. A number of labs in Finland, UK, USA, and Germany have generated mouse models of NCL diseases (*CLN1, CLN2, CLN3, CLN5, CLN6, CLN7, CLN8, CLN10*). Gene targeting technology has allowed selected mutations within a gene to be engineered, offering an experimental background more alike to that in human disease. Generally large numbers of experimental animals can be created, and survival is long enough for a high number of experiments to be carried out. This is the major advantage as compared to spontaneously occurring large mammals, where the number of animals carrying homozygous mutations for a selected gene is relatively low and experiments need to gather as much information as possible from each animal. Advances in technology allow engineered mice to be investigated according to the same methodologies described for large mammalian brains (for example imaging and neurophysiological studies).

Simple Cellular Systems

The availability of miniaturized technology has allowed the use of high throughput systems including invertebrate such as Zebrafish, Drosophila, the social amoeba Dictyostelium, yeast, *in vitro* cell systems, such as SH-SY5Y Neuroblastoma cells, and from human patient fibroblasts (obtained from skin biopsies). The use of these simple cellular models make them suitable to address selected questions and obtain results from targeted experiments which are easily repeatable and provide high statistical power (129).

Human Fibroblasts and Derived Cells

A major advantage of using human fibroblasts is the ability to transform them into neuronal cells through a complex process *in vitro* of diversion from the primary cell lineage, regression of the cells into induced pluripotent stem cells (iPSCs) and transformation into neurons using *ad-hoc* enriched media. Such studies are already in progress: neuronal cells, derived from fibroblasts of CLN5 patients, showed features consistent with a NCL phenotype (130), and transformed neurons, derived from fibroblasts of CLN2 and CLN3 patients, showed impaired activities of the lysosomal-mediated pathways (131).

A major limitation of this technology, however, is that fibroblasts carry the genetic background of the affected patient, so that direct comparisons between the phenotypic variations of a specific mutation among cell lines from different patients and inferences about the functional implications of specific mutations are challenging. This difficulty can be addressed by combining the CRISPR/Cas9 genome editing technology to generate targeted mutations in mammals as well as in healthy human iPSCs or in human embryonic stem cells (132, 133), without the bias of the potential effect of the mutated gene on the native genetic background.

A further development in the *in vitro* technology, using human cells to investigate the functional effects of individual mutations, is the generation from iPSCs of cerebral organoids (134). Such an approach allows investigation of the effects of a mutated gene not only on the mature cells, but also to evaluate its putative involvement in general neurodevelopmental mechanisms. The available technology makes it possible to test hypotheses multi-Omically, and therefore obtain information about the main pathways and functions modified by the mutated gene during the mini-brain development (135).

The Omics Approach: Aspects and Significance

Omics approaches represent recently developed technologies which provide high-throughput data related to the genome (DNA) the transcriptome (RNA) the proteome (protein), and the metabolome (metabolic products). The integrated study of data derived from Omics investigations represents the foundation of system biology, and it has been applied to investigate disease mechanisms, particularly the identification of affected biological pathways, which may become therapeutic targets as well as potential biomarkers (127). The new knowledge acquired from these methodologies is predicted to contribute to significant advances in the field of NCL research. The use of Omics technologies to NCLs is relatively recent; several issues have been addressed in both human and experimental animal models of several NCL forms. Major studies performed on tissues from patients affected with different NCL forms are summarized in (**Supplementary Table 2**).

Pathology and Pathogenetic Mechanisms

Neuronal death is the disease hallmark shared among all NCL forms: it affects CNS neurons, ganglionic neuronal cells of the retina and even ganglionic neurons of intramural ganglia of the bowel wall. All gray regions of the brain are affected by neuronal death, showing however differential patterns in the topography of neuronal loss, the rate of progression and the secondary involvement of the white matter. Selective neurodegeneration, targeting specific regions and particular cell populations, can be observed during the early stages of the disease, and the patterns of disease evolution can be monitored by neuroimaging studies (136–140). Whether these features reflect the genetic heterogeneity of the NCL is a matter to be investigated further. As clearly indicated by the clinical symptoms at onset and by the patterns of disease progression, cerebral and cerebellar cortices are the most affected brain regions in humans. Selective hippocampal pathology has also been described in different NCL (141). In some forms, particularly in CLN2 disease, the rate of atrophy of the cerebellar cortex is faster than observed in the telencephalic cortex. Less marked is the involvement of the basal ganglia in the early stages of the disease, but eventually generalized atrophy of all gray structures is observed in all childhood NCLs. The neuronal loss leads also to progressive atrophy of the centra semi-ovale, due to the lack of axonal projections from and to the cortex, which is accompanied by progressive enlargement of the ventricular system. Traditional human neuropathological studies have shown that neurons of the spinal cord are also affected in many NCL forms (1, 27, 142). These findings emphasize further the generalized susceptibility of all neuronal cells to this condition.

Cell Pathology

Most information concerning the selective involvement of gray structures and the neuronal loss in the NCL brain come from elegant studies carried out using mouse models, which allowed descriptions of the temporal evolution of neuronal loss, established the role of both astroglia and microglia in the brain pathology (143), dissected out the most vulnerable cell compartments involved in neuronal degeneration and proposed hypotheses regarding the cellular mechanisms and pathways underlying the progressive neuronal loss seen in the NCLs.

Topography of the Lesions

A gradient of neuronal degeneration is commonly observed, affecting the cortex, the cerebellum and the thalami. Thalamic gliosis seems to precede the onset of cortical involvement (neuronal loss) in both CLN1 and CLN10 mouse models (144) and early thalamic involvement is considered as a radiological marker of LSD (145). A reverse pattern of degeneration is observed in CLN5-/- mice (146). Recently, neuronal loss has also been described in in a mouse model of the disease in the spinal cord, preceding the neuronal loss of the remaining brain regions (147).

Such gradients of evolution are less evident in human post-mortem studies, in which all the brain structures are severely affected in a similar manner: neuropathological examinations are performed several years after the disease onset.

Selected Involvement of Neuronal Cell Structures

Neuronal death represents the end point of a complex process to which several processes contribute. However, some groups of neuronal cells seem to be more vulnerable to the pathological condition, as shown by some elegant experimental studies.

Axons are affected: early axonal breakdown was shown in the CLN1 KO mouse model (144); impaired elongation and branching, giving origin to a stunted growth was observed in vitro, in a neuronal-like cell system, overexpressing CLN1 gene (148).

Several aspects of synaptic pathology were also reported. Loss of synaptic proteins was reported in both mouse (CLN1) and ovine (CLN5) models of diseases (144, 149), as well as impaired synaptic vesicles recycling (150). Impaired NMDA-R development was described by Koster et al. (151). Recently, a reduction of functional voltage-gated Calcium channel, in differentiated SH-SY5Y cells, overexpressing CLN1/PPT1 gene was reported (152).

Results from different experimental techniques provide evidence that neuronal connectivity is affected in several NCL models. Impaired cellular function due to the distorted topology of the neuronal cells because of the intra-lysosomal storage is amplified by the impaired neuron-to-neuron communication, strongly contributing to neuronal dysfunction and subsequently cell death.

Pathogenetic Mechanisms

The molecular mechanisms leading to endo-lysosomal storage formation have not so far been fully elucidated. It should be noted that a primary defect of lysosomal proteolytic activity is present in only four NCL forms, whereas in the remaining NCLs, storage accumulation is associated with impaired cellular degradation of large molecules by means of a complex pathway, in which lysosomal hydrolytic enzymes are a major, but not exclusive, component. Likewise, which mechanisms link the formation of the endo-lysosomal storage and death of the neuronal cells remains unknown (38).

Apoptosis

Apoptosis was considered to be the main mechanism leading to cell death following the detection of targeted markers in canine, ovine and human brains and photoreceptors (153, 154). Subsequently, the role of autophagy was investigated in the NCLs, possibly because of the evidence of a temporal link between autophagy and apoptosis (155). Activation of unfolded protein response and apoptosis was shown in the brain of an early mouse model of CLN1 disease (156). The presence of apoptotic cells and expression of apoptosis markers along with lysosomal dysfunction and autophagic stress were also detected in the CNS of a mouse model of Cathepsin D deficiency (157).

Autophagy

The evidence of lysosomal accumulation of subunit c of the mitochondrial ATPase F0 complex suggested this occurred secondarily to impaired degradation pathways. Cao et al. (49) showed that *autophagy* was severely affected in a mouse model of CLN3 disease. Moreover, the detection of cell death following inhibition of autophagy suggested that activated autophagy represents a pro-survival response of the cell to the disease process.

Recently impaired autophagy was demonstrated to occur in different experimental models of a number of NCL forms and supported the increasing evidence that dysregulated autophagy is commonly detected in the LSDs (158).

Fibroblasts of patients mutated in *KCTD7* (associated with NCL14) had impaired autophagy (159). Impaired autophagy was also detected in a KO mouse model for CLN1 disease, which is associated to mutated palmitoyl-protein thioesterase-1 (PPT1), a lysosomal enzyme that catalyzes the deacylation of S-palmitoylated proteins. Such a defect was associated with impaired palmitoylation of a protein (Rab7), crucial for autophagosome-lysosome fusion, and therefore leading to impaired degradative function along the autophagic pathway (160).

A role for *CLN5* in dysregulating autophagy was recently suggested by studies in patient fibroblasts and *CLN5*-deficient HeLa cells which showed increased autophagy flux (161). Likewise aberrant development was reported in Dictyostelium deleted in *cln5*, homologous to human CLN5 gene in which autophagy seems to play a regulatory role in terminal differentiation of the amoeba (162).

Interestingly, none of the recently described four experimental conditions, related to mutations in genes associated with different NCL, and associated with impaired autophagy, are associated

with genes belonging to the autophagy degradation pathway. That implies that inhibited and/or dysregulated autophagy is the endpoint of a complex network of molecular interactions occurring in NCL neurons, whose outcome is the death of neuronal cells.

Oxidative Stress and the Mitochondrial Machinery

Along with investigations directed to the basic mechanism of cell death (such as apoptosis and autophagy), a large amount of data has been obtained over the last two decades from studies aimed at understanding more about the cell physiology in NCL, and other mechanisms which might hamper cell viability and therefore contribute to the cell death process.

Oxidative stress was investigated early, as it was also suggested by the clinical anecdotal observations of transient, worsening of the general wellbeing of affected children under energy-requiring circumstances (e.g. fever, general anesthesia).

Impaired activities of OxPhos enzymes were reported in CLN1 fibroblasts and its putative role on triggering apoptotic cascade was well established (163, 164). Moreover, structural abnormalities of the mitochondrial reticulum as well as abnormal ROS production were described in human fibroblasts (165, 166). More recently, a quantitative proteomic study showed impaired mitochondrial function in different human cells (knocked out in *CLN5*) models and in Cln5–/– mouse cerebral cortex. Impaired autophagy machinery coupled with mitophagy activation processes were observed linking the CLN5 protein to the process of neuronal death (37).

The involvement of the mitochondrial machinery is not surprising in neurodegeneration (as either primary or secondary event) because of the high dependance of neurons on energy supplied by the oxidative metabolism. These findings, however, reinforce the recent evidence of cross-talk between different cell compartments (including lysosomes and mitochondria, as well as endoplasmic reticulum) which seem to be affected differentially in the NCLs. Greater understanding of the molecular relationships of such intracellular "dialogues" might provide clues toward targeted treatments for specific NCL forms (35).

Immunomodulation and the Inflammatory Response

As in several neurodegenerative disorders (including Alzheimer's disease), inflammatory changes of the neuropil are significant in PPT-1 deficient mice, including the increased production of pro-cytokines, recruitment of inflammatory cells, microglia activation (167–169). The inflammatory response has therefore been considered a powerful amplifier of the NCL disease pathology. Such findings led to several trials using selected immunomodulatory drugs, which alleviated neurological symptoms in the affected animals, but were more effective if applied before the onset of symptomatology (170). The same rationale lead to a clinical trial for juvenile NCL patients (CLN3 disease), using mychophenolate, an immunosuppressant used off label for autoimmune neurological conditions. The drug was tolerated, but there is no evidence so far for a positive effect on clinical disease progression (171).

TREATMENT: THE STATE OF ART

General Issues

The medical management of children and young adults affected by one of the NCLs continues to be mainly symptomatic, delivered through a multidisciplinary and multiagency approach, working closely with family members and carers. Medical care should follow internationally agreed standards and guidelines for individual symptoms and organ systems (for example epilepsy, respiratory, orthopedic and gut) and be delivered in line with the holistic values of palliative care. These approaches with particular reference to the NCLs have been described in a number of publications in recent years (108, 109, 120). Experts in rare diseases should be cognisant of advances in these areas or at least be able to signpost families and carers to appropriate expertise. Some advances have been mentioned in previous sections of this review. In particular, advances in management of chest symptoms in children with complex neurodisability and dependence, including for example use of cough assist devices and non-invasive ventilation, together with cardiac pacing suggest sick sinus syndrome. Newer tone management and anti-seizure treatment modalities (including drugs, dietary therapies and stimulation techniques) should be considered and discussed openly when considering the goals of care and potential medical interventions with families.

Epilepsy and Tone/Movement Disorder Management

Perhaps the most troubling clinical symptoms are seizures throughout the disease course and tone/movement disorder management in the later stages.

These are widely reported by parents to be the most worrying and by professionals as the most challenging. Many NCL forms of early childhood onset are characterized by a progressive myoclonic epilepsy syndrome whereas in CLN3 disease with juvenile onset generalized motor and absence seizures predominate. Clinical experience suggests that anti-seizure medications considered best for generalized genetic epilepsies are most effective in these disorders whereas those which are known to exacerbate myoclonus are best avoided. The most commonly used anti-seizure medications are valproate, levetiracetam and the benzodiazepines in varying combinations. Carbamazepine is avoided. Lamotrigine is reported to be helpful in combination with valproate in the later stages of CLN2 disease and is very effective at high doses in older children and young adults with CLN3 disease. Experience of international clinical experts varies but there is consensus that medications for epilepsy are used to alleviate seizure burden rather than with the goal of complete seizure freedom and that drug combinations are usually necessary. In some patients the seizure burden is less at the late stages of the disease and medication can be reduced. Ketogenic diet is not contraindicated, is often very well tolerated and may also be helpful.

Movement disorders are increasingly recognized in the NCLs. In several NCL forms with onset in the preschool or early school years (CLN2, CLN5, CLN6, CLN7, CLN8) a choreo-athetosis or mixed movement disorder with dystonia becomes evident a

TABLE 4 | Selected therapeutic trials (completed and on-going).

Disease	Therapy	Study	Via	Vector/Product	Clinical trial code[#]
CLN2	ERT*	Phase 1/2 open label Safety and efficacy	i.c.v.	Recombinant protein	NCT01907087
CLN2	ERT*	Open label Long term efficacy	i.c.v.	Recombinant protein	NCT02485899
CLN2	GRT*	Safety	i.c.	AAVrh.10CUhCLN2	NCT01161576
CLN3	GRT	Phase 1/2 open-label	i.t.	AT-GTX-502	NCT03770572
CLN6	GRT	Phase 1/2 open-label	i.t.	AT-GTX-501	NCT02725580
CLN6	GRT	Long term efficacy	None	AT-GTX-501-01	NCT04273243
CLN7	GRT	Phase 1 open-label	i.t.	AAV9/CLN7	NCT04737460
CLN3	SRT	Phase 1/2	os	BBDF 101	None

*ERT, Enzyme Replacement Therapy; GRT, Gene Replacement Therapy; SRT, Substrate Reduction Therapy; i.c.v., intracerebralventricular injection; i.c., intracerebral (burr holes) injection; i.t., intrathecal injection. *completed studies. [#]ClinicalTrials.gov.*

few years after symptom onset. Determining which involuntary movements are ictal and which are movement disorder can be challenging and EEG video telemetry with event capture can be very helpful. The movement disorder progresses to spasticity in the late stages of all NCL forms, complicated by spinal scoliosis and joint contractures. Baclofen, trihexyphenidyl, clonidine and botulinum toxin injections are used extensively and in combination.

Gabapentin has proven very useful for irritability and distress in the rapidly deteriorating phase of CLN2 disease.

Orphan Drugs and New Treatments

We are beginning to recognize new clinical phenotypes for the few NCL types now amenable to disease modifying therapies such as enzyme replacement and gene therapy. These treatment approaches are new and the full range of implications on symptoms and quality of life as well as survival and longevity is yet to be established. It may well be that there should be a shift from a mainly palliative approach for symptom control to more aggressive intervention with the expectation of complete symptom control (for example seizure freedom) in treated individuals. Close working with the patients themselves, families, caregivers and family representative organizations will be crucial as we go forward to define what standard care should look like.

In LSD and more recently in NCL therapeutic strategies have emerged with the aim of preventing abnormal storage formation and/or to deplete the abnormal endo-lysosomal accumulation, with the ultimate goal to reduce and/or stop the disease progression (122, 172). Several challenges must be overcome, for example the route of drug delivery to the CNS, safety, outcomes and how best to measure efficacy, and above all the still incomplete knowledge of the patho-mechanisms underlying each NCL form. This is still the age of a "gross" therapeutic approach, using replacement therapies (the mutated gene or the missing enzyme), whereas more classical pharmacological treatments targeting crucial steps of the important metabolic pathways still lag behind. It is likely that a combined approach will be necessary to achieve the good therapeutic outcomes patients, families and professionals would like to see.

Complementation Therapy

Complementation therapies (either as gene replacement therapy or enzyme replacement therapy, ERT) are becoming available for some NCL forms (**Table 4**). ERT became available as part of a clinical trial almost a decade ago for CLN2 disease. Following the decision of the regulatory agencies (FDA and EMA), the recombinant lysosomal enzyme, cerliponase alpha, replacing the ineffective gene product has been commercially available since 2017 (Brineura©). The enzyme is administered directly to the CNS via an intra cerebro-ventricular catheter every 2 weeks. The safety of this approach is well documented by the most expert group with this procedure (173). As for its efficacy there is good evidence that disease progression is slowed down over a 4 year period, as compared with historical untreated controls (174, 175). Likewise, slowed disease progression was observed in a small cohort of CLN2 children (assessed for a shorter period of time), who received intracerebral injection of adenovirus expressing *CLN2* (176). Even more interesting results were reported in a very small group of pre-school children who underwent treatment before or a few months after clinical onset of the disease, with delayed onset of disease symptoms as well as maintenance of early cognitive skills (177, 178).

There are ongoing phase 1/2 open label studies to evaluate the safety and efficacy of gene therapy administered intrathecally for CLN3 disease, CLN6 disease and CLN7 disease. These studies have not yet been completed, and the final results are not available (**Table 4**).

Substrate Reduction Therapy

Some preliminary data indicate lysosomal glycosphingolipid accumulation in CLN3 and CLN5 disease. Such findings have suggested an approach using prevention of abnormal lysosomal storage, by interfering with glucosylceramide and ganglioside production using Miglustat a glucosylceramide synthase inhibitor, which is currently used in Niemann-Pick type C disease. This drug is authorized in Europe (but not in USA) for the disease, and beneficial effects of this treatment on juvenile- or adult-onset N-PC were reported on a large cohort of patients (179). Recently it was also shown that trehalose (a disaccharide)

promotes lysosomal clearance in storage disease by activation of TFEB, a transcription factor involved in lysosome biogenesis and recruitment (180). By combining the potential benefits of Miglustat and the physiological role of Trehalose a phase 1/2 study is planned using a small number of CLN3 patients using a new recently FDA approved drug (BBDF 101) which contains both components.

Storage Dissolution

There was only one study so far, aiming to deplete the endo-lysosomal storage in CLN1 disease by the combined action of phosphocystemaine (which cleaves thioester linkage in palmitoylated protein and N-acetylcysteine a strong anti-oxidant which also cleaves thiosterer linkage). The long term outcome showed only minor subjective benefits for patients, along with some improvements related to EEG pattern and storage dissolution (181).

CONCLUDING REMARKS

NCL are rare, genetically determined, progressive diseases affecting several mammalian species. In humans they affect pre-school and school aged children (or more rarely they start in adulthood) and are defined by clinical criteria supported by pathological features. Amaurosis, seizures, ataxia, behavioral problems, cognitive decline are the major symptoms leading within a relatively short time span to dementia, loss of motor autonomy, blindness and dependence on caregivers. Autofluorescence and selected ultrastructural features of endo-lysosomal storage are the pathological markers detectable both in central and autonomic neurons as well as in several peripheral cells following skin biopsy. Modern diagnosis relies mainly on biochemical (for the NCLs caused by lysosomal enzyme deficiencies) and genetic studies.

Over last two decades dramatic advances in the knowledge of the molecular basis of NCL has been achieved in both humans and several experimental models, but the precise patho-mechanisms leading to cell pathology and neuronal death have not yet been fully elucidated. Nevertheless, a few targeted treatments for the NCLs have become available for human study recently, including enzyme replacement and gene therapies, although only one product is commercially available at present. New pharmacological approaches are foreseen in the near future. The safety and efficacy of such novel treatments are still under scrutiny; there is a conscious effort to ascertain whether new clinical phenotypes may arise as a consequence of these innovative treatments, as recently described in SMA type 1 (182). International collaborations are necessary, recruiting larger cohorts of patients, to generate robust natural history studies to support the regulatory approval process for new drugs (92, 183) and to inform health service delivery.

There is an urgent need to find safe and effective treatments for rare neurodegenerative diseases, such as the NCLs, and mutual agreements between patients, families and advocacy groups, the Health Systems and the pharmaceutical companies are mandatory. With such endeavors, challenges have become evident and will need to be overcome. Examples include ownership and sharing of personal data (natural history data and pre-marketing clinical data), the costs of treatment, the health benefit/cost ratio. These issues are particularly relevant in Europe, where publically funded health systems have to protect and guarantee the privacy of clinical data and, at the same time, have to balance the costs of novel treatments for rare diseases against wider health targets and to guarantee equitable access without discrimination.

AUTHOR CONTRIBUTIONS

AS developed the original concept and design of this study. Both authors have made substantial contributions to further modifications of the work, were actively involved in the revision of this manuscript, approved the submitted version and agreed to be personally accountable for the author's own contributions and to ensure any part of the work are appropriately investigated, documented in accordance with the literature, and read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Mole SE, Williams RE, Goebel HH. *The Neuronal Ceroid Lipofuscinoses (Batten Disease)*. New york: Oxford University Press. (2011). doi: 10.1093/med/9780199590018.001.0001
2. Stengel OC. Account of a singular illness among four siblings in the vicinity of Roraas. In: *Ceroid Lipofuscinosis (Batten Disease),* eds Armstrong D, Koppang N, Rider JA. (Amsterdam: Elsevier/North Holland Biomedical Press). (1826/1982). p. 17–19.
3. Sachs B. A family form of idiocy, generally fatal and associated with early blindness (amaurotic familial idiocy). *NY Med J.* (1896) 63:697–703.
4. Tay W. Symmetrical changes in the region of the yellow spot in each eye of an infant. *Trans Ophtalm Soc.* (1881) 1:55–7.
5. Batten FE. Family cerebral degeneration with macular change (so-called juvenile form of family amaurotic idiocy). *Q J Med.* (1914) 7:444–54.
6. Bielschowski M. Zur Histopathologie und Pathogenese der amaurotischen Idiotie mit besonderer Berucksichtigung der zerebellaren Veranderungen. *J Psychol Neurol.* (1920) 25:123–99.
7. Jansky J. Dosud nepopsany pripad familiarni amauroticke idiotie komplikovane s hypoplasii mozeckovou. *Sborn Lek.* (1908) 13:165–96. doi: 10.1111/j.1471-0528.1908.tb14524.x

8. Spielmeyer W. Klinische und amaurotische Untersuchungen uber eine besondere Form von familiarer amaurotischer Idiotie. *Histol und Histopathol.* (1908) 2:193–251.
9. Vogt H. Familiare amaurotiche Idiotie. Histologische und histopatologischie. *Studien Archiv Kinderheilkd.* (1909) 51:1–35.
10. Kufs H. Uber eine Spatform der amaurotischen Idiotie und ihre heredofamiliaren Grundlagen. *Z Ges Neurol Psychiatr.* (1925) 195:165–88. doi: 10.1007/BF02900989
11. Schaffer K. Beitrage zur Nosographie und Histopathologie der amaurotisch-familiaren Idiotieformen. *Arch Psychiatr Nervenkr.* (1906) 42:127–60. doi: 10.1007/BF02046807
12. Batten FE, Mayou MS. Family cerebral degeneration with macular changes. *Proc R Soc Med.* (1915) 8:70–90. doi: 10.1177/0035915715008 01624
13. Sjogren T. Die juvenile amaurotische Idiotie. Klinische und erblichkeitsmedizinische Untersuchungen. *Hereditas.* (1931) 14:197–426. doi: 10.1111/j.1601-5223.1931.tb02535.x
14. Klenk E. Beitrage zur Chemie der Lipidosen, Niemann-Pickschen Krankheit und amaurotischen Idiotie. *Hoppe-Seyler Z Physiol Chem.* (1939) 262:128–43. doi: 10.1515/bchm2.1939.262.3-5.128
15. Zeman W, Donohue S. Fine structure of the lipid bodies in juvenile amaurotic idiocy. *Acta Neuropathol.* (1963) 3:144–9. doi: 10.1007/BF00687063
16. Zeman W. The neuronal ceroid lipofuscinoses. In: *Progress in Neuropathology.* ed. *Zimmermann HM. (New York: Grune Stratton).* (1976). p. 207–223.
17. Berkovic SF, Carpenter S, Andermann F, Andermann E, Wolfe SE. Kufs' disease: a critical reappraisal. *Brain.* (1988) 111:27–62. doi: 10.1093/brain/111.1.27
18. Zeman W, Dyken P. Neuronal ceroid-lipofuscinosis (Batten's disease): relationship to amaurotic family idiocy? *Pediatrics.* (1969) 44:570–83. doi: 10.1542/peds.44.4.570
19. Svennerholm L. The chemical structure of normal brain and Tay-Sachs gangliosides. *Biochem Biophys Res Commun.* (1962) 9:436–41. doi: 10.1016/0006-291X(62)90030-X
20. Seehafer SS, Pearce DA. You say lipofuscin, we say ceroid: Defining autofluorescent storage material. *Neurobiol Aging.* (2006) 27:576–88. doi: 10.1016/j.neurobiolaging.2005.12.006
21. Palmer DN, Fearnley IM, Walker JE, Hall NA, Lake BD, Wolfe LS, et al. Mitochondrial ATP synthase subunit c storage in the ceroid-lipofuscinoses (Batten disease). *Am J Med Genet.* (1992) 42:561–7. doi: 10.1002/ajmg.1320420428
22. Tyynela J, Palmer DN, Baumann M, Haltia M. Storage of saposins A and D in infantile neuronal ceroid lipofuscinosis. *FEBS Lett.* (1993) 330:8–12. doi: 10.1016/0014-5793(93)80908-D
23. Anderson G, Goebel HH, Simonati A. Human pathology of NCL. *Bioch Biophys Acta.* (2013) 1832:1807–26. doi: 10.1016/j.bbadis.2012.11.014
24. Brett EM, Lake BD. Reassessment of rectal approach to neuropathology in childhood. *Arch Dis Child.* (1975) 50:753–762. doi: 10.1136/adc.50.10.753
25. Simonati A, Rizzuto N. Neuronal Ceroidolipofuscinoses: Pathological features of bioptic specimens in 28 patients. *Neurol Sci.* (2000) 21:S63–70. doi: 10.1007/s100720070042
26. Wang SH, Williams EM, Takahash K, Nelvagal HR, et al. Enteric Nervous System defects underlie bowel dysfunction in Cln1, Cln2 and Cln3 disease mice: a new therapeutic target? *In: NCL2021 October 6-10th abstract book, (abstract* 013) (2021). p 27.
27. Haltia M. The neuronal ceroid lipofuscinoses. *J Neuropathol Exp Neurol.* (2003) 62:1–13. doi: 10.1093/jnen/62.1.1
28. Haltia M, Goebel HH. The neuronal ceroid-lipofuscinoses: a historical introduction. *Biochim Biophys Acta.* (2013) 1832:1795–800. doi: 10.1016/j.bbadis.2012.08.012
29. Hersheson J, Burke D, Clayton R, Anderson G, Jacques TS, Mills MP, et al. Cathepsin D deficiency causes juvenile onset ataxia and distinctive muscle pathology. *Neurology.* (2014) 83:1873–5. doi: 10.1212/WNL.00000000000 00981
30. Kohlschutter A, Williams RE, Goebel HH, Mole SE, Boustany RM, van Diggelen OP, et al. NCL Diagnosis and Algorithms. In: *The Neuronal Ceroid Lipofuscinoses (Batten Disease).*, eds. Mole SE, Williams RE, Goebel HH. *(New York: Oxford University Press).* (2011). p. 24–34. doi: 10.1093/med/9780199590018.003.0003
31. Vesa J, Hellsten E, Verkruyse LA, Camp LA, Rapola J, Santavuori P, et al. Mutations in the palmitoyl protein thioesterase gene causing infantile neuronal ceroid lipofuscinosis. *Nature.* (1995) 376:584–7. doi: 10.1038/376584a0
32. The International Batten Disease Consortium. Isolation of a novel gene underlying Batten disease, CLN3. *Cell.* (1995) 82:949–57. doi: 10.1016/0092-8674(95)90274-0
33. Williams RE, Mole SE. New nomenclature and classification scheme for the neuronal ceroid lipofuscinoses. *Neurology.* (2012) 79:183–91. doi: 10.1212/WNL.0b013e31825f0547
34. Gardner E, Mole SE. The genetic basis of phenotypic heterogeneity in the neuronal ceroid lipofuscinoses. *Front Neurol.* (2021) 12:754045. doi: 10.3389/fneur.2021.754045
35. Bajaj L, Sharma J, di Ronza A, Zhang P, Eblimit A, Pal R, et al. A CLN6-CLN8 complex recruits lysosomal enzymes at the ER for Golgi transfer. *J Clin Invest.* (2020) 130:4118–32. doi: 10.1172/JCI130955
36. Di Ronza A, Bajaj L, Sharma J, Sanagasetti D, Lotfi P, Adamski CJ, et al. CLN8 is an endoplasmic reticulum cargo receptor that regulates lysosome biogenesis. *Nat Cell Biol.* (2018) 20:1370–7. doi: 10.1038/s41556-018-0228-7
37. Doccini S, Morani F, Nesti C, Pezzini F, Calza G, Soliymani R, et al. Proteomic and functional analyses in disease models reveal CLN5 protein involvement in mitochondrial dysfunction. *Cell Death Discovery.* (2020) 6:18. doi: 10.1038/s41420-020-0250-y
38. Mukherjee AB, Appu AP, Sadhukhan T, Casey S, Mondal A, Zhang Z, et al. Molecular Neurodegeneration. Emerging new roles of the lysosome and neuronal ceroid lipofuscinoses. *Mol Neurodegen.* (2019) 14:4. doi: 10.1186/s13024-018-0300-6
39. Katz ML, Coates JR, Sibigtroth CM, Taylor JD, Carpentier M, Young WM, et al. Enzyme replacement therapy attenuates disease progression in a canine model of late-infantile neuronal ceroid lipofuscinosis (CLN2 disease). *J Neurosci Res.* (2014) 92:1591–8. doi: 10.1002/jnr.23423
40. Magrinelli F, Pezzini F, Moro F, Santorelli FM, Simonati A. Diagnostic methods and emerging treatments for adult neuronal ceroid lipofuscinosis (Kufs disease). *Expert Opinion in Orphan Drugs.* (2017) 5:487–501. doi: 10.1080/21678707.2017.1325359
41. Arsov T, Smith KR, Damiano J, Franceschetti S, Canafoglia L, Bromhead CJ, et al. Kufs disease, the major adult form of neuronal ceroid lipofuscinosis, caused by mutations in CLN6. *Am J Hum Genet.* (2011) 88:1–8. doi: 10.1016/j.ajhg.2011.04.004
42. Nosková L, Stránecký V, Hartmannová H, Pristoupilová A, Barešová V, Ivánek R, et al. Mutations in DNAJC5, encoding cysteine-string protein alpha, cause autosomal-dominant adult-onset neuronal ceroid lipofuscinosis. *Am J Hum Genet.* (2011) 89:241–252. doi: 10.1016/j.ajhg.2011.07.003
43. Velinov M, Dolzhanskaya N, Gonzalez M, Powell E, Konidari I, Hulme W, et al. Mutations in the gene DNAJC5 cause autosomal dominant Kufs disease in a proportion of cases: study of the Parry family and 8 other families. *PLoS ONE.* (2012) 7:e29729. doi: 10.1371/journal.pone.0029729
44. Smith KR, Damiano J, Franceschetti S, Carpenter S, Canafoglia L, Morbin M, et al. Strikingly different clinicopathological phenotypes determined by progranulin-mutation dosage. *Am J Hum Genet.* (2012) 90:1102–7. doi: 10.1016/j.ajhg.2012.04.021
45. Smith KR, Dahl HH, Canafoglia L, Andermann E, Damiano J, Morbin M, et al. Cathepsin F mutations cause type B Kufs disease, an adult-onset neuronal ceroid lipofuscinosis. *Hum Mol Genet.* (2013) 22:1417–23. doi: 10.1093/hmg/dds558
46. Qureshi YH, Patel VM, Berman DE, Kothiya MJ, Neufeld JL, Vardarajan B, et al. An Alzheimer's disease-linked loss-of-function cln5 variant impairs cathepsin d maturation, consistent with a retromer trafficking defect. *Mol Cell Biol.* (2018) 38:e00011–18. doi: 10.1128/MCB.00011-18
47. Geier EG, Bourdenx M, Storm NJ, Cochran JN, Sirkis DW, Hwang JH, et al. Rare variants in the neuronal ceroid lipofuscinosis gene MFSD8 are candidate risk factors for frontotemporal dementia. *Acta Neuropathol.* (2019) 137:71–88. doi: 10.1007/s00401-018-1925-9

48. Bras J, Verloes A, Schneider SA, Mole SE, Guerreiro RJ. Mutation of the parkinsonism gene ATP13A2 causes neuronal ceroid-lipofuscinosis. *Hum Mol Genet.* (2012) 21:2646–2650. doi: 10.1093/hmg/dds089
49. Cao Y, Espinola JA, Fossale E, Massey AC, Cuervo AM, MacDonald ME, et al. Autophagy is disrupted in a knock-out mouse model of juvenile neuronal ceroid lipofuscinosis. *J Biol Chem.* (2006) 281:20483–93. doi: 10.1074/jbc.M602180200
50. Thelen M, Damme M, Schweizer M, Hagel C, Wong AMS, Cooper JD, et al. Disruption of the autophagy-lysosome pathway is involved in neuropathology of the nclf mouse model of neuronal ceroid lipofuscinosis. *PLoS ONE.* (2012) 7:e35493. doi: 10.1371/journal.pone.0035493
51. Giorgi C, Bouhamida E, Danese A, Previati M, Pinton P, Patergnani S. Relevance of autophagy and mitophagy dynamics and markers in neurodegenerative diseases. *Biomedicines.* (2021) 9:149. doi: 10.3390/biomedicines9020149
52. Root J, Merino P, Nuckols A, Johnson M, Kukar T. Lysosome dysfunction as a cause of neurodegenerative diseases: Lessons from frontotemporal dementia and amyotrophic lateral sclerosis. *Neurobiol Dis.* (2021) 154:105360. doi: 10.1016/j.nbd.2021.105360
53. Sleat DE, Donnelly RJ, Lackland H, Liu CG, Sohar I, Pullarkat RK, et al. Association of mutations in a lysosomal protein with classical late-infantile neuronal ceroid lipofuscinosis. *Science.* (1997) 277:1802–5. doi: 10.1126/science.277.5333.1802
54. van Diggelen OP, Keulemans JL, Winchester B, Hofman IL, Vanhanen SL, Santavuori P, et al. A rapid fluorogenic palmitoyl-protein thioesterase assay: pre- and postnatal diagnosis of INCL. *Mol Genet Metab.* (1999) 66:240–4. doi: 10.1006/mgme.1999.2809
55. Maeser S, Brindusa-Alina P, Ion L, Rawer S, Kohlschutter A, Santorelli FM, et al. Enzymatic diagnosis of neuronal ceroid lipofuscinoses in dried blood specimens using substrates for concomitant tandem mass spectrometry and fluorimetry. *J Mass Spectrom.* (2021) 56:4675. doi: 10.1002/jms.4675
56. Savukoski M, Klockars T, Holmberg V, Santavuori P, Lander ES, Peltonen L. CLN5, a novel gene encoding a putative transmembrane protein mutated in Finnish variant late infantile neuronal ceroid lipofuscinosis. *Nat Genet.* (1998) 19:286–8. doi: 10.1038/975
57. Ranta S, Zhang Y, Ross B, Lonka L, Takkunen E, Messer A, et al. The neuronal ceroid lipofuscinoses in human EPMR and mnd mutant mice are associated with mutations in CLN8. *Nat Genet.* (1999) 23:233–6. doi: 10.1038/13868
58. Teixeira CA, Espinola J, Huo L, Kohlschütter J, Persaud Sawin DAP, Minassian B, et al. Novel mutations in the CLN6 gene causing a variant late infantile neuronal ceroid lipofuscinosis. *Hum Mutat.* (2003) 21:502–8. doi: 10.1002/humu.10207
59. Williams RE. Appendix 1: NCL incidence and prevalence data. In: *The Neuronal Ceroid Lipofuscinoses (Batten Disease).*, eds Mole SE, Williams RE, and Goebel HH. (New York: Oxford University Press) (2011) p. 361–365. doi: 10.1093/med/9780199590018.003.0023
60. Santorelli FM, Garavaglia B, Cardona F, Nardocci N, Dalla Bernardina B, Sartori S, et al. Molecular epidemiology of childhood neuronal ceroid-lipofuscinosis in Italy. *Orphanet J Rare Dis.* (2013) 8:19. doi: 10.1186/1750-1172-8-19
61. Simpson NA, Wheeler ED, Pearce DA. Screening, diagnosis and epidemiology of Batten disease. *Expert Opinion in Orphan Drug.* (2014) 2:903–10. doi: 10.1517/21678707.2014.935762
62. Sleat DE, Gedvilaite E, Zhang Y, Lobel P, Xing J. Analysis of large-scale whole exome sequencing data to determine the prevalence of genetically-distinct forms of neuronal ceroid lipofuscinosis. *Gene.* (2016) 593:284–91. doi: 10.1016/j.gene.2016.08.031
63. Mole SE, Williams RE, Goebel HH. Correlations between genotype, ultrastructural morphology and clinical phenotype in the neuronal ceroid lipofuscinoses. *Neurogenetics.* (2005) 6:107–26. doi: 10.1007/s10048-005-0218-3
64. Cotman SL, Karaa A, Staropoli JF, Sims KB. Neuronal Ceroid Lipofuscinosis: Impact of recent genetic advances and expansion of the clinicopathologic spectrum. *Curr Neurol Neurosci Rep.* (2013) 13:366. doi: 10.1007/s11910-013-0366-z
65. Mink JW, Augustine EF, Adams HR, Marshall FJ, Kwon JM. Classification and natural history of the neuronal ceroid lipofuscinoses. *J Child Neurol.* (2013) 28:1101–5. doi: 10.1177/0883073813494268
66. Schulz A, Kohlschütter A, Mink J, Simonati A, Williams RE. NCL diseases - clinical perspectives. *Bioch Biophys Acta.* (2013) 1832:1801–6. doi: 10.1016/j.bbadis.2013.04.008
67. Simonati A, Pezzini F, Moro F, Santorelli FM. Neuronal Ceroid Lipofuscinosis: the increasing spectrum of an old disease. *Curr Mol Med.* (2014) 8:1043–51. doi: 10.2174/1566524014666141010154913
68. Nita AD, Mole SE, Minassian BA. Neuronal ceroid lipofuscinoses. *Epileptic Disord.* (2016) 18:S73–88. doi: 10.1684/epd.2016.0844
69. Specchio N, Ferretti A, Trivisano M, Pietrafusa N, Pepi C, Calabrese C, et al. Neuronal Ceroid Lipofuscinosis: Potential for Targeted Therapy. *Drugs.* (2021) 81:101–23. doi: 10.1007/s40265-020-01440-7
70. Steinfeld R, Heim P, von Gregory H, Meyer K, Ullrich K, Goebel HH, et al. Late infantile neuronal ceroid lipofuscinosis: quantitative description of the clinical course in patients with CLN2 mutations. *Am J Med Genet.* (2002) 112:347–54. doi: 10.1002/ajmg.10660
71. Marshall FJ, de Blieck EA, Mink JW, Dure L, Adams HR, Messing S, et al. A clinical rating scale for Batten disease: reliable and relevant for clinical trials. *Neurology.* (2005) 65:275–9. doi: 10.1212/01.wnl.0000169019.41332.8a
72. Masten MC, Williams JD, Vermilion J, Adams HR, Vierhile A, Collins A, et al. The CLN3 Disease Staging System: A new tool for clinical research in Batten disease. *Neurology.* (2020) 94:e2436–40. doi: 10.1212/WNL.0000000000009454
73. Varvagiannis K, Hanquinet S, Billieux MH, De Luca R, Rimensberger P, Lidgren M, et al. Congenital neuronal ceroid lipofuscinosis with a novel CTSD gene mutation: a rare cause of neonatal-onset neurodegenerative disorder. *Neuropediatrics.* (2018) 49:150–3. doi: 10.1055/s-0037-1613681
74. Vanhanen S-L, Sainio K, Lappi M, Santavuori P. EEG and evoked potentials in infantile ceroid-lipofuscinosis. *Dev Med Child Neurol.* (1997) 39:456–63. doi: 10.1111/j.1469-8749.1997.tb07465.x
75. Simonati A, Tessa A, Dalla Bernardina B, Biancheri R, Veneselli E, Tozzi G, et al. Variant late infantile neuronal ceroidlipofuscinosis because of *CLN1* mutations. *Pediatr Neurol.* (2009) 40:271–6. doi: 10.1016/j.pediatrneurol.2008.10.018
76. Doccini S, Sartori S, Maeser S, Pezzini F, Rossato S, Moro F, et al. Early infantile Neuronal Ceroid Lipofuscinosis (CLN10 Disease) associated with a novel mutation in CTSD. *J Neurol.* (2016) 263:1029–1032. doi: 10.1007/s00415-016-8111-6
77. Pampiglione G, Harden A. Neurophysiological identification of a late infantile form of 'neuronal lipidosis'. *J Neurol Neurosurg Psychiatry.* (1973) 36:68–74. doi: 10.1136/jnnp.36.1.68
78. Specchio N, Bellusci M, Pietrafusa N, Trivisano M, de Palma L, Vigevano F. Photosensitivity is an early marker of neuronal ceroid lipofuscinosis type 2 disease. *Epilepsia.* (2017) 58:1380–8. doi: 10.1111/epi.13820
79. Peña JA, Cardozo JJ, Montiel CM, Molina OM, Boustany R-M. Serial MRI findings in the Costa Rican variant of neuronal ceroid-lipofuscinosis. *Pediatr Neurol.* (2001) 25:78–80. doi: 10.1016/S0887-8994(01)00284-3
80. Berkovic SF, Oliver KL, Canafoglia L, Krieger P, Damiano JA, Hildebrand MS, et al. Kufs disease due to mutation of CLN6: clinical, pathological and molecular features. *Brain.* (2019) 142:59–69. doi: 10.1093/brain/awy297
81. Augustine EF, Adams HR, Beck CA, Vierhile A, Kwon JM, Rothberg PG, et al. Standardized assessment of seizures in patients with juvenile neuronal ceroid lipofuscinosis. *Dev Med Child Neurol.* (2015) 57:366–71. doi: 10.1111/dmcn.12634
82. Arntsen V, Strandheim J, Helland IB, Sand T, Brodtkorb E. Epileptological aspects of juvenile neuronal ceroid lipofuscinosis (CLN3 disease) through the lifespan. *Epilepsy Behav.* (2019) 94:59–64. doi: 10.1016/j.yebeh.2019.02.020
83. Ostergaard JR. Gait phenotype in Batten disease: a marker of disease progression. *Eur J Pediatr Neurol.* (2021) 35:1–7. doi: 10.1016/j.ejpn.2021.09.004
84. Simonati A, Santorum E, Tessa A, Polo A, Simonetti F, Dalla Bernardina B, et al. A CLN2 nonsense mutation is associated with severe caudate atrophy and dystonia. *Neuropediatrics.* (2000) 31:199–201. doi: 10.1055/s-2000-7453

85. Saini AG, Sankhyan N, Singhi P. Chorea in late infantile neuronal ceroid lipofuscinosis: an atypical presentation. *Pediatr Neurol.* (2016) 60:75–8. doi: 10.1016/j.pediatrneurol.2016.02.015
86. Nickel M, Simonati A, Jacoby D, Lezius S, Kilian D, Van de Graaf B, et al. Disease characteristics and progression in patients with late infantile neuronal ceroid lipofuscinosis type 2 (CLN2) disease: an observational cohort study. *Lancet Child Adolesc Health.* (2018) 2:582–90. doi: 10.1016/S2352-4642(18)30179-2
87. Adams HR, Mink JW. Neurobehavioral features and natural history of juvenile neuronalceroid lipofuscinosis (Batten disease). *J Child Neurol.* (2013) 28:1128–36. doi: 10.1177/0883073813494813
88. Ostergaard JR. Juvenile neuronal ceroid lipofuscinosis (Batten disease): current insights. *Degener Neurol Neuromuscul Dis.* (2016) 6:73–83. doi: 10.2147/DNND.S111967
89. Adams HR, Beck CA, Levy E, Jordan R, Kwon JM, Marshall FJ, et al. Genotype does not predict severity of behavioural phenotype in juvenile neuronal ceroid lipofuscinosis (Batten disease). *Dev Med Child Neurol.* (2010) 52:637–43. doi: 10.1111/j.1469-8749.2010.03628.x
90. Cialone J, Adams HR, Augustine EF, Marshall FJ, Kwon JM, Newhouse N, et al. Females experience a more severe disease course in Batten disease. *J Inherit Metab Dis.* (2012) 35:549–55. doi: 10.1007/s10545-011-9421-6
91. Xin W, Mullen TE, Kiely R, Min J, Feng X, Cao Y, et al. CLN5 mutations are frequent in juvenile and late-onset non-Finnish patients with NCL. *Neurology.* (2010) 74:565–71. doi: 10.1212/WNL.0b013e3181cff70d
92. Simonati A, Williams RE, Nardocci N, Laine M, Battini R, Schulz A, et al. Disease natural history and phenotypic profiles in a cohort of patients affected with variant late infantile ceroid-lipofuscinosis 5 (CLN5). *Dev Med Child Neurol.* (2017) 58:815–21. doi: 10.1111/dmcn.13473
93. Lourenço CM, Pessoa A, Mendes CC, Rivera-Nieto C, Vergara D, Troncoso M, et al. Revealing the clinical phenotype of atypical neuronal ceroid lipofuscinosis type 2 disease: Insights from the largest cohort in the world. *J Paediatr Child Health.* (2021) 57:519–25. doi: 10.1111/jpc.15250
94. Ouseph MM, Kleinman ME, Wang QJ. Vision loss in juvenile neuronal ceroid lipofuscinosis (Batten disease). *Ann N Y Acad Sci.* (2016) 1371:55–67. doi: 10.1111/nyas.12990
95. Kovacs KD, Patel S, Orlin A, Kim K, Van Everen S, Conner T, et al. Symmetric age association of retinal degeneration in patients with CLN2-associated batten disease. *Ophthalmol Retina.* (2020)· 4:728–36. doi: 10.1016/j.oret.2020.01.011
96. Weleber RG, Gupta N, Trzupek KM, Wepner MS, Kurz DE, Milam AH. Electroretinographic and clinicopathologic correlations of retinal dysfunction in infantile neuronal ceroid lipofuscinosis (infantile Batten disease). *Mol Genet Metab.* (2004) 83:128–37. doi: 10.1016/j.ymgme.2004.06.019
97. Khan KN, El-Asrag ME, Ku CA, Holder GE, McKibbin M, Arno G, et al. Specific alleles of CLN7/MFSD8, a protein that localizes to photoreceptor synaptic terminals, cause a spectrum of nonsyndromic retinal dystrophy. *Invest Ophthalmol Vis Sci.* (2017) 58:2906–14. doi: 10.1167/iovs.16-20608
98. Kuper WFE, van Alfen C, Rigterink RH, Fuchs SA, van Genderen MM, van Hasselt PM. Timing of cognitive decline in CLN3 disease. *J Inherit Metab Dis.* (2018) 41:257–61. doi: 10.1007/s10545-018-0143-x
99. Bauwens M, Storch S, Weisschuh N, Ceuterick-de Groote C, De Rycke R, Guillemyn B, et al. Functional characterization of novel MFSD8 pathogenic variants anticipates neurological involvement in juvenile isolated maculopathy. *Clin Genet.* (2020) 97:426–436. doi: 10.1111/cge.13673
100. Chin JJ, Behnam B, Davids M, Sharma P, Zein WM, Wang C, et al. Novel mutations in CLN6 cause late-infantile neuronal ceroid lipofuscinosis without visual impairment in two unrelated patients. *Mol Genet Metab.* (2019) 126:188–95. doi: 10.1016/j.ymgme.2018.12.001
101. Lin T-Y, Chang Y-C, Hsiao Y-R, Chien Y, Cheng Y-C, Wu J-R, et al. Identification of novel genomic-variant patterns of OR56A5, OR52L1, and CTSD in retinitis pigmentosa patients by whole-exome sequencing. *Int J Mol Sci.* (2021) 22:5594. doi: 10.3390/ijms22115594
102. Wang F, Wang H, Tuan H-F, Nguyen DH, Sun V, Keser V, et al. Next generation sequencing-based molecular diagnosis of retinitis pigmentosa: Identification of a novel genotypephenotype correlation and clinical refinements. *Hum Genet.* (2014) 133:331–345. doi: 10.1007/s00439-013-1381-5
103. Murray SJ, Russell KN, Melzer TR, Gray SJ, Heap SJ, Palmer DN, 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
104. Whiting RE, Kick GR, Ota-Kuroki J, Lim S, Castaner LJ, Jensen CA, et al. Intravitreal enzyme replacement inhibits progression of retinal degeneration in canine CLN2 neuronal ceroid lipofuscinosis. *Exp Eye Res.* (2020) 198:108135. doi: 10.1016/j.exer.2020.108135
105. Kirveskari E, Partinen M, Salmi T, Sainio K, Telakivi T, Hämäläinen M, et al. Sleep alterations in juvenile neuronal ceroid-lipofuscinosis. *Pediatr Neurol.* (2000) 22:347–54. doi: 10.1016/S0887-8994(00)00138-7
106. Kirveskari E, Partinen M, Santavuori P. Sleep and its disturbance in a variant form of late infantile neuronal ceroid lipofuscinosis (CLN5). *J Child Neurol.* (2001) 16:707–13. doi: 10.1177/088307380101601001
107. Lehwald LM, Pappa R, Steward S, de Los Reyes E. Neuronal ceroid lipofuscinosis and associated sleep abnormalities. *Pediatr Neurol.* (2016) 59:30–5. doi: 10.1016/j.pediatrneurol.2016.02.009
108. Augustine EF, Adams HR, de Los Reyes E, Drago K, Frazier M, Guelbert N, et al. Management of CLN1 disease: international clinical consensus. *Paediatr Neurol.* (2021) 120:38–51. doi: 10.1016/j.pediatrneurol.2021.04.002
109. Mole SE, Schulz A, Badoe E, Berkovic SF, de Los Reyes EC, Dulz S, et al. Guidelines on the diagnosis, clinical assessments, treatment and management for CLN2 disease patients. *Orphanet J Rare Dis.* (2021) 16:185. doi: 10.1186/s13023-021-01813-5
110. Hätönen T, Kirveskari E, Heiskala H, Sainio K, Laakso ML, Santavuori P. Melatonin ineffective in neuronal ceroid lipofuscinosis patients with fragmented or normal motor activity rhythms recorded by wrist actigraphy. *Mol Genet Metab.* (1999) 66:401–6. doi: 10.1006/mgme.1999.2815
111. Staropoli JF, Xin W, Barone R, Cotman SL, Sims KB. An atypical case of neuronal ceroid lipofuscinosis with co-inheritance of a variably penetrant POLG1 mutation. *BMC Med Genet.* (2012) 13:50. doi: 10.1186/1471-2350-13-50
112. Sun Y, Almomani R, Breedveld GJ, Santen GW, Aten E, Lefeber DJ, et al. Autosomal recessive spinocerebellar ataxia 7 (SCAR7) is caused by variants in TPP1, the gene involved in classic late-infantile neuronal ceroid lipofuscinosis 2 disease (CLN2 Disease). *Hum Mutat.* (2013) 34:706–13. doi: 10.1002/humu.22292
113. Di Giacopo R, Cianetti L, Caputo V, La Torraca I, Piemonte F, Ciolfi A, et al. Protracted late infantile ceroid lipofuscinosis due to TPP1 mutations: Clinical, molecular and biochemical characterization in three sibs. *J Neurol Sci.* (2015) 356:65–71. doi: 10.1016/j.jns.2015.05.021
114. Platt FM, d'Azzo A, Davidson BL, Neufeld EF, Tifft CJ. Lysosomal storage diseases. *Nat Rev Dis Primers.* (2018) 4:27. doi: 10.1038/s41572-018-0025-4
115. Østergaard JR, Rasmussen TB, Mølgaard H. Cardiac involvement in juvenile neuronal ceroid lipofuscinosis (Batten disease). *Neurology.* (2011) 76:1245–51. doi: 10.1212/WNL.0b013e31821435bd
116. Ofman IL, Van Der Wal AC, Dingemans KP, Becker AE. Cardiac pathology in neuronal ceroid lipofuscinoses-a clinicopathologic correlation in three patients. *Eur J Paediatr Neurol.* (2001) 5:213–7. doi: 10.1053/ejpn.2000.0465
117. Fukumura S, Saito Y, Saito T, Komaki H, Nakagawa E, Sugai K, et al. Progressive conduction defects and cardiac death in late infantile neuronal ceroid lipofuscinosis. *Dev. Med. Child Neurol.* (2021) 54:663–666. doi: 10.1111/j.1469-8749.2011.04170.x
118. Augestad LB, Flanders WD. Occurrence of and mortality from childhood neuronal ceroid lipofuscinosis in Norway. *J Child Neurol.* (2006) 21:917–22. doi: 10.1177/08830738060210110801
119. Siintola E, Partanen S, Strömme P, Haapanen A, Haltia M, Maehlen J, et al. Cathepsin D deficiency underlies congenital human neuronal ceroid-lipofuscinosis. *Brain.* (2006) 129:1438–45. doi: 10.1093/brain/awl107
120. Williams RE, Adams HR, Blohm M, Cohen-Pfeffer JL, de Los Reyes E, Denecke J, et al. Management Strategies for CLN2 Disease. *Pediatr Neurol.* (2017) 69:102–12. doi: 10.1016/j.pediatrneurol.2017.01.034
121. Gissen P, Specchio N, Olaye A, Jain M, Butt T, Ghosh W, et al. Investigating health-related quality of life in rare diseases: a case study in utility value determination for patients with CLN2 disease (neuronal ceroid lipofuscinosis type 2). *Orphanet J Rare Dis.* (2021) 16:217. doi: 10.1186/s13023-021-01829-x

122. Mole SE, Anderson G, Band HA, Berkovic SF, Cooper JD, Kleine Holthaus SM, et al. Clinical challenges and future therapeutic approaches for neuronal ceroid lipofuscinosis. *Lancet Neurol.* (2019) 18:107–16. doi: 10.1016/S1474-4422(18)30368-5
123. Nelvagal HR, Lange J, Takahashi K, Tarczyluk-Wells MA, Cooper JD. Pathomechanisms in the neuronal ceroid lipofuscinoses. *Biochim Biophys Acta Mol Basis Dis.* (2020) 1866:165570. doi: 10.1016/j.bbadis.2019.165570
124. Sawiak SJ, Perumal SR, Rudiger SR, Matthews L, Mitchell NL, McLaughlan CJ, et al. Rapid and progressive regional brain atrophy in CLN6 Batten disease affected sheep measured with longitudinal magnetic resonance imaging. *PLoS ONE.* (2015) 10:e0132331. doi: 10.1371/journal.pone.0132331
125. Perentos N, Martins AQ, Watson TC, Bartsch U, Mitchell NL, Palmer DN, et al. Translational neurophysiology in sheep: measuring sleep and neurological dysfunction in CLN5 Batten disease affected sheep. *Brain.* (2015) 138:862–74. doi: 10.1093/brain/awv026
126. Russell KN, Mitchell NL, Anderson NG, Bunt CR. Computed tomography provides enhanced techniques for longitudinal monitoring of progressive intracranial volume loss associated with regional neurodegeneration in ovine neuronal ceroid lipofuscinoses. *Brain Behav.* (2018) 8:e01096. doi: 10.1002/brb3.1096
127. Kline RA, Wishart TM, Mills K, Heywood WE. Applying modern Omic technologies to the Neuronal Ceroid Lipofuscinoses. *Biochim Biophys Acta Mol Basis Dis.* (2020) 1866:165498. doi: 10.1016/j.bbadis.2019.06.012
128. Mitchell NL, Russell KN, Wellby MP, Wicky HE, Schoderboeck L, Barrell GK, 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) 26:2366-78. doi: 10.1016/j.ymthe.2018.07.015
129. Minnis CJ, Thornton CD, FitzPatrick LM, Tristan R, McKay TR. Cellular models of Batten disease. *Biochim Biophys Acta Mol Basis Dis.* (2020) 1866:165559. doi: 10.1016/j.bbadis.2019.165559
130. Uusi-Rauva K, Blom T, Schantz-Fant V, Blom T, Jalanko A, Kyttälä A. Induced pluripotent stem cells derived from a CLN5 patient manifest phenotypic characteristics of neuronal ceroid lipofuscinoses. *Int J Mol Sci.* (2017) 18:955. doi: 10.3390/ijms18050955
131. Lojewski X, Staropoli JF, Biswas-Legrand S, Simas AM, Haliw L, Selig MK, et al. Human iPSC models of neuronal ceroid lipofuscinosis capture distinct effects of TPP1 and CLN3 mutations on the endocytic pathway. *Hum Mol Genet.* (2014) 23:2005–22. doi: 10.1093/hmg/ddt596
132. Eaton SL, Proudfoot C, Lillico SG, Skehel P, Kline RA, Hamer K, et al. CRISPR/Cas9 mediated generation of an ovine model for infantile neuronal ceroid lipofuscinosis (CLN1 disease). *Sci Rep.* (2019) 9:9891. doi: 10.1038/s41598-019-45859-9
133. Ma L, Prada AM, Schmidt M, Morrow EM. Generation of pathogenic TPP1 mutations in human stem cells as a model for neuronal ceroidlipofuscinosis type 2 disease. *Stem Cell Res.* (2021) 53:102323. doi: 10.1016/j.scr.2021.102323
134. Chiaradia I, Lancaster MA. Brain organoids for the study of human neurobiology at the interface of *in vitro* and *in vivo*. *Nat Neurosci.* (2020) 23:1496–508. doi: 10.1038/s41593-020-00730-3
135. Gomez-Giro G, Arias-Fuenzalida J, Jarazo J, Zeuschner D, Ali M, Possemis N, et al. Synapse alterations precede neuronal damage and storage pathology in a human cerebral organoid model of CLN3-juvenile neuronal ceroid lipofuscinosis. *Acta Neuropathol Commun.* (2019) 7:222. doi: 10.1186/s40478-019-0871-7
136. Autti TH, Hämäläinen J, Mannerkoski M, Van Ven Leemput K, Aberg LE. JNCL patients show marked brain volume alterations on longitudinal MRI in adolescence. *J Neurol.* (2008) 255:1226–30. doi: 10.1007/s00415-008-0891-x
137. Baker EH, Levin SW, Zhang Z, Mukherjee AB. MRI brain volume measurements in infantile neuronal ceroid lipofuscinosis. *AJNR Am J Neuroradiol.* (2017) 38:376–82. doi: 10.3174/ajnr.A4978
138. Dyke JP, Sondhi D, Voss HU, Yohay K, Hollmann C, Mancenido D, et al. Brain region-specific degeneration with disease progression in late infantile neuronal ceroid lipofuscinosis (CLN2 Disease). *AJNR Am J Neuroradiol.* (2016) 37:1160–9. doi: 10.3174/ajnr.A4669
139. Löbel U, Sedlacik J, Nickel M, Lezius S, Fiehler J, Nestrasil I, et al. Volumetric description of brain atrophy in Neuronal Ceroid Lipofuscinosis 2: supratentorial gray matter shows uniform disease progression. *AJNR Am J Neuroradiol.* (2016) 37:1938–43. doi: 10.3174/ajnr.A4816
140. Roine T, Roine U, Tokola A, Balk MH, Mannerkoski M, Åberg L, et al. Topological alterations of the structural brain connectivity network in children with juvenile neuronal ceroid lipofuscinosis. *AJNR Am J Neuroradiol.* (2019) 40:2146–53. doi: 10.3174/ajnr.A6306
141. Tyynelä J, Cooper JD, Khan MN, Shemilt SJ, Haltia M. Hippocampal pathology in the human neuronal ceroid-lipofuscinoses: distinct patterns of storage deposition, neurodegeneration and glial activation. *Brain Pathol.* (2004) 14:349–57. doi: 10.1111/j.1750-3639.2004.tb00077.x
142. Radke J, Stenzel W, Goebel HH. Human NCL neuropathology. *Biochim Biophys Acta.* (2015) 1852:2262–6. doi: 10.1016/j.bbadis.2015.05.007
143. Lange J, Haslett LJ, Lloyd-Evans E, Pocock JM, Sands MS, Williams BP, et al. Compromised astrocyte function and survival negatively impact neurons in infantile neuronal ceroid lipofuscinosis. *Acta Neuropathol Commun.* (2018) 6:74. doi: 10.1186/s40478-018-0575-4
144. Kielar C, Wishart TM, Palmer A, Dihanich S, Wong AM, Macauley SL, et al. Molecular correlates of axonal and synaptic pathology in mouse models of Batten disease. *Hum Mol Genet.* (2009) 18:4066–80. doi: 10.1093/hmg/ddp355
145. Autti T, Joensuu R, Aberg J. Decreased T2 signal. in the thalami. may be a sign of lysosomal storage disease. *Neuroradiology.* (2007) 49:571–8. doi: 10.1007/s00234-007-0220-6
146. von Schantz C, Kielar C, Hansen SN, Pontikis CC, Alexander NA, Kopra O, et al. Progressive thalamocortical neuron loss in Cln5 deficient mice: distinct effects in Finnish variant late infantile NCL. *Neurobiol Dis.* (2009) 34:308–19. doi: 10.1016/j.nbd.2009.02.001
147. Shyng C, Nelvagal HR, Dearborn JT, Tyynelä J, Schmidt RE, Sands MS, et al. Synergistic effects of treating the spinal cord and brain in CLN1 disease. *Proc Natl Acad Sci U S A.* (2017) 114:E5920–E5929. doi: 10.1073/pnas.1701832114
148. Pezzini F, Bettinetti L, Di Leva F, Bianchi M, Zoratti E, Carrozzo R, et al. Transcriptomic profiling discloses molecular and cellular events related to neuronal differentiation in SH-SY5Y neuroblastoma cells. *Cell Mol Neurobiol.* (2017) 37:665–82. doi: 10.1007/s10571-016-0403-y
149. Amorim IS, Mitchell NL, Palmer DN, Sawiak SJ, Mason R, Wishart TM, et al. Molecular neuropathology of the synapse in sheep with CLN5 Batten disease. *Brain Behav.* (2015) 5:e00401. doi: 10.1002/brb3.401
150. Kim SJ, Zhang Z, Sarkar C, Tsai PC, Lee YC, Dye L, et al. Palmitoyl protein thioesterase-1 deficiency impairs synaptic vesicle recycling at nerve terminals, contributing to neuropathology in humans and mice. *J Clin Invest.* (2008) 118:3075–86. doi: 10.1172/JCI33482
151. Koster KP, Francesconi W, Berton F, Alahmadi S, Srinivas R, Yoshii A. Developmental NMDA receptor dysregulation in the infantile neuronal ceroid lipofuscinosis mouse model. *Elife.* (2019) 8:e40316. doi: 10.7554/eLife.40316
152. Demontis GC, Pezzini F, Margari E, Bianchi M, Longoni B, Doccini S, et al. Electrophysiologic profile remodeling via selective suppression of voltage-gated currents by CLN1/PPT1 over expression in human neuronal-like cells. *Front Cell Neurosci.* (2020) 14:569598 doi: 10.3389/fncel.2020.569598
153. Lane SC, Jolly RD, Schmechel DE, Alroy J, Boustany RM. Apoptosis as the mechanism of neurodegeneration in Batten's disease. *J Neurochem.* (1996) 67:677–83. doi: 10.1046/j.1471-4159.1996.67020677.x
154. Hachiya Y, Hayashi M, Kumada S, Uchiyama A, Tsuchiya K, Kurata K. Mechanisms of neurodegeneration in neuronal ceroid-lipofuscinoses. *Acta Neuropathol.* (2006) 111:168–77. doi: 10.1007/s00401-005-0024-x
155. Boya P, González-Polo RA, Casares N, Perfettini JL, Dessen P, Larochette N, et al. Inhibition of macroautophagy triggers apoptosis. *Mol Cell Biol.* (2005) 25:1025–40. doi: 10.1128/MCB.25.3.1025-1040.2005
156. Zhang Z, Lee YC, Kim SJ, Choi MS, Tsai PC, Xu Y, et al. Palmitoyl-protein thioesterase-1 deficiency mediates the activation of the unfolded protein response and neuronal apoptosis in INCL. *Hum Mol Genet.* (2006) 15:337–46. doi: 10.1093/hmg/ddi451

157. Shacka JJ, Klocke BJ, Young C, Shibata M, Olney JW, Uchiyama Y, et al. Cathepsin D deficiency induces persistent neurodegeneration in the absence of Bax-dependent apoptosis. *J Neurosci.* (2007) 27:2081–90. doi: 10.1523/JNEUROSCI.5577-06.2007
158. Seranova E, Connolly KJ, Zatyka M, Rosenstock TR, Barrett T, Tuxworth RI, et al. Dysregulation of autophagy as a common mechanism in lysosomal storage diseases. *Essays Biochem.* (2017) 61:733–49. doi: 10.1042/EBC20170055
159. Metz KA, Teng X, Coppens I, Lamb HM, Wagner BE, Rosenfeld JA, et al. KCTD7 deficiency defines a distinct neurodegenerative disorder with a conserved autophagy-lysosome defect. *Ann Neurol.* (2018) 84:766–80. doi: 10.1002/ana.25351
160. Sarkar C, Sadhukhan T, Bagh MB, Appu AP, Chandra G, Mondal A, et al. Cln1-mutations suppress Rab7-RILP interaction and impair autophagy contributing to neuropathology in a mouse model of infantile neuronal ceroid lipofuscinosis. *J Inherit Metab.* (2020) 43:1082–101. doi: 10.1002/jimd.12242
161. Adams J, Feuerborn M, Molina JA, Wilden AR, Adhikari B, Budden T, et al. Autophagy-lysosome pathway alterations and alpha-synuclein up-regulation in the subtype of neuronal ceroid lipofuscinosis, CLN5 disease. *Sci Rep*. (2019) 9:151. doi: 10.1038/s41598-018-36379-z
162. McLaren MD, Mathavarajah S, Kim WD, Yap SQ, Huber RJ. Aberrant autophagy impacts growth and multicellular development in a dictyostelium knockout model of CLN5 disease. *Front Cell Dev Biol.* (2021) 9:657406. doi: 10.3389/fcell.2021.657406
163. Jolly RD, Brown S, Das AM, Walkley SU. Mitochondrial dysfunction in the neuronal ceroidlipofuscinoses (Batten disease). *Neurochem Internat*. (2002) 40:565–71. doi: 10.1016/S0197-0186(01)00128-0
164. Wei H, Kim SJ, Zhang Z, Tsai PC, Wisniewski KE, Mukherjee AB. ER and oxidative stresses are common mediators of apoptosis in both neurodegenerative and non-neurodegenerative lysosomal storage disorders and are alleviated by chemical chaperones. *Hum Mol Genet*. (2008) 17:469–77. doi: 10.1093/hmg/ddm324
165. Pezzini F, Gismondi F, Tessa A, Tonin P, Carrozzo R, Mole SE, et al. Involvement of the mitochondrial compartment in human NCL fibroblasts. *Biochem Biophys Res Commun.* (2011) 416:159–64. doi: 10.1016/j.bbrc.2011.11.016
166. Vidal-Donet JM, Cárcel-Trullols J, Casanova B, Aguado C, Knecht E. Alterations in ROS activity and lysosomal pH account for distinct patterns of macroautophagy in LINCL and JNCL fibroblasts. *PLoS ONE.* (2013) 8:e55526. doi: 10.1371/journal.pone.0055526
167. Macauley SL, Wong AM, Shyng C, Augner DP, Dearborn JT, Pearse Y, et al. An anti-neuroinflammatory that targets dysregulated glia enhances the efficacy of CNS-directed gene therapy in murine infantile neuronal ceroid lipofuscinosis. *J Neurosci.* (2014) 34:13077–82. doi: 10.1523/JNEUROSCI.2518-14.2014
168. Groh J, Kühl TG, Ip CW, Nelvagal HR, Sri S, Duckett S, et al. Immune cells perturb axons and impair neuronal survival in a mouse model of infantile neuronal ceroid lipofuscinosis. *Brain.* (2013) 136:1083–101. doi: 10.1093/brain/awt020
169. Sadhukhan T, Bagh MB, Appu AP, Mondal A, Zhang W, Liu A, et al. In a mouse model of INCL reduced S-palmitoylation of cytosolic thioesterase APT1 contributes to microglia proliferation and neuroinflammation. *J Inherit Metab Dis.* (2021) 44:1051–69. doi: 10.1002/jimd.12379
170. Groh J, Berve K, Martini R. Immune modulation attenuates infantile neuronal ceroid lipofuscinosis in mice before and after disease onset. *Brain Commun.* (2021) 3:fcab047. doi: 10.1093/braincomms/fcab047
171. Augustine EF, Beck CA, Adams HR, Defendorf S, Vierhile A, Timm D, et al. Short-term administration of mycophenolate is well-tolerated in CLN3 disease (juvenile neuronal ceroid lipofuscinosis). *JIMD Rep*. (2019) 43:117–24. doi: 10.1007/8904_2018_113
172. Geraets RD, yon Koh S, Hastings ML, Kielian T, Pearce DA, Weimer JM. Moving towards effective therapeutic strategies for Neuronal Ceroid Lipofuscinosis. *Orphanet J Rare Dis.* (2016) 11:40. doi: 10.1186/s13023-016-0414-2
173. Schwering C, Kammler G, Wibbeler E, Christner M, Knobloch JK, Nickel M, et al. Development of the "Hamburg best practice guidelines for ICV-enzyme replacement therapy (ERT) in CLN2 disease" based on 6 years treatment experience in 48 patients. *J Child Neurol.* (2021) 36:635–41. doi: 10.1177/0883073821989154
174. Schulz A, Ajayi T, Specchio N, de Los Reyes E, Gissen P, Ballon D, et al. Study of intraventricular cerliponase alfa for CLN2 Disease. *N Engl J Med.* (2018) 378:1898–907. doi: 10.1056/NEJMoa1712649
175. Kohlschutter A, Schulz A, Bartsch U, Storch S. Current and emerging treatment strategies for neuronal ceroid lipofuscinoses. *CNS Drugs.* (2019) 33:315–25. doi: 10.1007/s40263-019-00620-8
176. Sondhi D, Kaminsky SM, Hackett NR, Pagovich OE, Rosenberg JB, De BP, 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:eabb5413. doi: 10.1126/scitranslmed.abb5413
177. Schaefers J, van der Giessen LJ, Klees C, Jacobs EH, Sieverdink S, Dremmen MHG, et al. Presymptomatic treatment of classic late-infantile neuronal ceroid lipofuscinosis with cerliponase alfa. *Orphanet J Rare Dis*. (2021) 16:221. doi: 10.1186/s13023-021-01858-6
178. Estublier B, Cano A, Hoebeke C, Pichard S, Scavarda D, Desguerre I, et al. Cerliponase alfa changes the natural history of children with neuronal ceroid lipofuscinosis type 2: the first French cohort. *Eur J Paediatr Neurol.* (2021) 30:17–21. doi: 10.1016/j.ejpn.2020.12.002
179. Patterson MC, Mengel E, Vanier MT, Schwierin B, Muller A, Cornelisse P, et al. Stable or improved neurological manifestations during miglustat therapy in patients from the international disease registry for Niemann-Pick disease type C: an observational cohort study. *Orphanet J Rare Dis*. (2015) 10:65. doi: 10.1186/s13023-015-0284-z
180. Bajaj L, Lotfi P, Pal R, Ronza AD, Sharma J, Sardiello M. Lysosome biogenesis in health and disease. *J Neurochem.* (2019) 148:573–89. doi: 10.1111/jnc.14564
181. Levin SW, Baker EH, Zein WM, Zhongjian Z, Quezado ZMNC, Ning M, et al. Oral cysteamine bitartrate and N-acetylcysteine for patients with infantile neuronal ceroid lipofuscinosis: a pilot study. *Lancet Neurol.* (2014) 13:777–87. doi: 10.1016/S1474-4422(14)70142-5
182. Masson R, Brusa C, Scoto M, Baranello, G. Brain, cognition and language development in spinal muscular atrophy type 1: a scoping review. *Dev. Med. Child Neurol*. (2021) 63:527–36. doi: 10.1111/dmcn.14798
183. Nickel M, Schulz A. Natural history studies in NCL and their expanding role in drug development - experiences from CL2 disease and relevance for clinical trials. *Front Neurol*. (2022). [in press].

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The LINCE Project: A Pathway for Diagnosing NCL2 Disease

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Introduction: Neuronal Ceroid Lipofuscinosis (NCL) comprises a clinically and genetically heterogeneous group of 13 neurodegenerative lysosomal storage disorders. Neuronal Ceroid lipofuscinosis type 2 disease (NCL2), caused by the deficient lysosomal enzyme tripeptidyl peptidase 1 (TPP1), is the only one with an approved enzyme replacement treatment (ERT). Early initiation of ERT appears to modify significantly the natural history of the disease. We aimed to shorten the time to diagnosis of NCL2.

Methods: In March 2017, we started per first time in Spain a selective screening program, the LINCE project, in pediatric patients with clinical symptoms compatible with NCL2 disease. The program covered the whole country. We distributed kits to pediatricians with the necessary material to assess patients. All samples in this study were received within one week of collection. Enzymatic activity determined on dried blood spots was the main method used to screen for TPP1 and palmitoyl protein thioesterase 1 (PPT1) for the differential diagnosis with neuronal ceroid lipofuscinosis type 1 (NCL1).

Results: Over a period of three years, we received 71 samples. The analysis was minimally invasive, relatively cheap and fast-executing. Three cases identified as a direct result of the selective screening strategy were confirmed by genetic study of NCL2 disease with a median age of 4.5 years. Our screening method has a specificity of 100%, and, with the absence to date of false negatives. We did not detect any NCL1-positive cases.

Conclusions: LINCE proved to be a simple, useful, and reliable tool for the diagnosis of NCL2, enabling clinicians to diagnose NCL2 faster. The presence of NCL2-positive cases in our population and availability of treatment may facilitate the inclusion of NCL2 in neonatal screening programs for early diagnosis.

Keywords: NCL2 disease, dried blood spot, early diagnosis, enzymatic activity, screening tripeptidyl peptidase 1

INTRODUCTION

Neuronal Ceroid Lipofuscinoses (NCLs, otherwise known as Batten disease) comprise a group of progressive encephalopathies that typically present during childhood and are characterized by intralysosomal accumulation of an autofluorescent lipopigment, lipofuscin, in various tissues. All except one (NCL4B) have an autosomal recessive inheritance pattern. Although NCLs are the most common cause of neurodegeneration during infancy and childhood, they are rare even as a combined group.

Since they were first described in 1903 by the British pediatrician and neurologist Frederick Batten, NCLs were originally classified according to the age of onset of clinical symptoms into four categories: infantile (NCL1), late infantile (NCL2), juvenile (NCL3), and adult (NCL4) (1).

NCLs are currently classified numerically according to their gene defect, and 13 different forms have been described (**Table 1**), with an incidence that varies between 0.6 and 14/100,000 newborns in different populations (2). Most forms are characterized by progressive motor and intellectual deterioration, seizures, blindness, and early death.

NCL2 (Jansky-Bielschowsky disease; MIM #204500) is one of the most frequent NCLs and is caused by autosomal recessive mutations in the TPP1 gene resulting in a deficiency of the lysosomal enzyme tripeptidyl peptidase 1 (TPP1). Clinical manifestations start between 2 and 4 years of age, with epilepsy that becomes resistant to multiple antiepileptic drugs, myoclonic ataxia, pyramidal signs, and developmental regression (3–5). The disease progresses rapidly in most patients between the ages of ∼3 and 6 years and in very quick successive stages after the onset of symptoms. Life expectancy does not go beyond adolescence. Biochemically, a defect in TPP1 (6–8), a pepstatin-insensitive protease (9) leads to an accumulation of undegraded lipofuscin causing massive neuronal cell atrophy and death (10) and resulting in brain and retinal degeneration. Historically, diagnosis of NCLs was based on clinical features and the presence of ultrastructural lysosomal inclusions of various types (3, 11, 12). Nowadays, diagnosis relies on biochemical and molecular analysis.

Prompt diagnosis is critical for optimal disease management and appropriate genetic counseling because of rapid disease progression after the onset of the first symptoms (13, 14). However, early detection is very challenging owing to poor awareness of the disease and the unspecific nature of the initial symptoms, which include language delay, motor difficulties, and epilepsy (15, 16), which can be associated with other diseases such as mucopolysaccharidoses, gangliosidoses, mucolipidoses, peroxisomal disorders, mitochondrial disorders and leukodystrophies (13). In fact, the delay between the onset of symptoms and the definitive diagnosis may span from 2 to 3 years (16).

Medical management has relied on symptomatic treatment and supportive and palliative care. Fortunately, since 2017, intraventricular enzyme replacement therapy (ERT) with cerliponase alfa has been approved in the USA by the Food and Drug Administration and in Europe by the European Medical Agency for the treatment of NCL2 (17–19). Early administration of cerliponase alfa has led to a significant reduction in the rate of decline of motor and language functions (20), thus delaying disease progression. Prompt diagnosis is mandatory prior to irreversible brain damage. Here, we present a screening strategy to facilitate rapid and reliable diagnosis of NCL2.

METHODS

Study Design

In March 2017, the Unit for the Diagnosis and Treatment of Congenital Metabolic Diseases at our Center started the

TABLE 1 | Classification of neuronal ceroid lipofuscinoses (according to the MIM database).

Disease	Phenotype MIM	Gene	Location	Inheritance	Protein product	Age at onset
NCL1	256730	*PPT1*	1p34.2	AR	**PPT1 (soluble lysosomal protein)**	Variable (Infantile)
NCL2	204500	*TPP1*	11p15.4	AR	**TPP1 (soluble lysosomal protein)**	Variable (Late Infantile)
NCL3	204200	*CLN3*	16p12.1	AR	Lysosomal membrane protein	Juvenile
NCL4A	204300	*CLN6*	15q23	AR	ER membrane protein	Adult
NCL4B	162350	*DNAJC5*	20q13.33	AD	Cytosolic, associated with vesicular membranes	Adult
NCL5	256731	*CLN5*	13q22.3	AR	Soluble lysosomal protein	Variable (Late Infantile)
NCL6	601780	*CLN6*	15q23	AR	ER membrane protein	Variable (Late Infantile)
NCL7	610951	*MFSD8*	4q28.2	AR	Lysosomal membrane protein	Variable (Late Infantile/Juvenile)
NCL8	600143 610003*	*CLN8*	8p23.3	AR	ER membrane protein	Variable (Late Infantile/Juvenile)
NCL10	610127	*CTSD*	11p15.5	AR	**CtsD (soluble lysosomal protein)**	Congenital
NCL11	614706	*GRN*	17q21.31	AR	Soluble lysosomal protein	Adult
NCL13	615362	*CTSF*	11q13.2	AR	**CtsF (soluble lysosomal protein)**	Adult
NCL14	611726	*KCTD7*	7q11.21	AR	Cytosolic, partially associated with membranes	Infantile

Available enzyme assays are shown in bold.

**Northern epilepsy variant; NCL9 (609055)—not molecularly characterized.*

PPT1, palmitoyl protein thioesterase 1; TPP1, tripeptidyl peptidase 1; Cts, cathepsin; AR, autosomic recessive; AD, autosomic dominant; ER, endoplasmic reticulum.

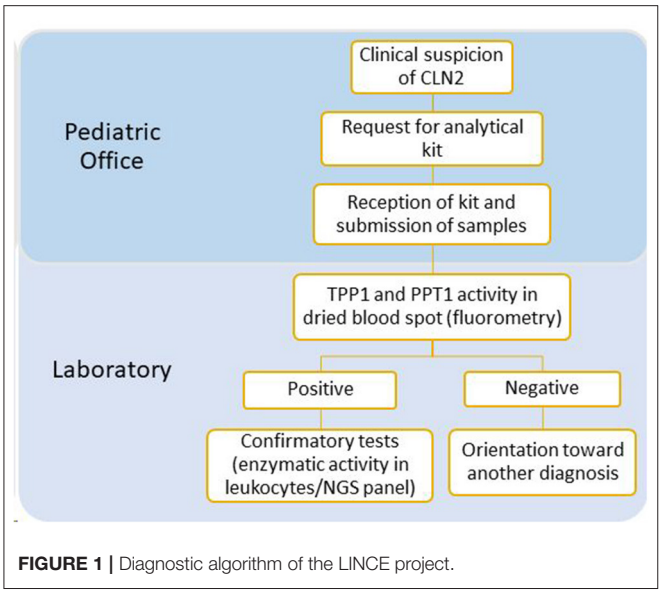

FIGURE 1 | Diagnostic algorithm of the LINCE project.

LINCE project with the endorsement of the Spanish Federation for Rare Diseases (FEDER) and the Spanish Society of Pediatric Neurology (SENEP) to identify possible cases of NCL2 throughout Spain. This study has been approved by the Territorial Research Ethics Committee with the register number 2017/185.

LINCE is a selective screening program aimed at pediatric patients (0–15 years) with clinical signs and symptoms compatible with NCL2 disease. Specific kits were designed with the necessary material, as follows: WhatmanTM 903 analytical paper for the collection of dried blood spots (DBS), an informed consent form to be signed by the parents of the participating children, a guide with indications on how to collect the samples, a contact telephone number, a contact e-mail address, and a clinical guide showing the signs and symptoms to be considered (**Supplementary Figure 1**).

Pediatricians interested in participating contacted us via email in order to obtain the LINCE kit. After reception, the enzymatic activities of TPP1 and palmitoyl protein thioesterase 1 (PPT1) were measured (**Figure 1**).

Phenotype

This nationwide selective screening project targeted an at-risk pediatric population, defined as patients whose pediatricians observed clinical signs and/or symptoms compatible with a diagnosis of NCL2. The alert to the main symptoms was taken into account, including: early language delay, other developmental delays as motor difficulties or ataxia; seizures including generalized tonic-clonic, absence, myoclonic, atonic, clonic and tonic, febrile seizures; myoclonic seizures, both epileptic and non-epileptic; dramatic loss of previously attained skills as loss of voluntary movements and ability to walk and language regression; movement disorders such as myoclonus, spasticity, dystonia and chorea; visual deterioration, blindness. Samples were received with a brief description of the patient's symptoms. All probands were phenotyped using human phenotype ontology (HPO) terms extracted from the records of the medical history sent.

Reagents

4-Methylumbelliferone sodium salt (4-MU), 7-amino-4-methylcoumarin (4-MC), 4-MU-β-D-galactoside, dimethyl sulfoxide, Triton X-100, DL-dithiothreitol, sodium azide, β-glucosidase, pepstatin A, E64 (trans-epoxysuccinyl-L-leucylamido (4-guanidino) butane), and ethylenediamine were purchased from Sigma-Aldrich Corp. (St. Louis, MO. USA). 4-MU-6-thio-palmitate-β-D-glucopyranoside was purchased from Carbosynth (United Kingdom). Ala-Ala-Phe-7-amido-4-methylcoumarin was purchased from Bachem (United Kingdom). Sodium carbonate, glycine, sodium hydroxide, acetic acid, sodium acetate, citric acid, sodium phosphate, sodium chloride, chloroform, and methanol were from Merck (Darmstadt, Germany). Bovine serum albumin (BSA) was purchased from ICN Biomedicals (Aurora, OH, USA). Ethylenediaminetetraacetic acid (EDTA) was purchased from Panreac (Barcelona).

Enzymatic Tests

As main screening method, enzymatic activity was determined on DBS for TPP1 (EC 3.4.14.9) and PPT1 (EC 3.1.2.22) for the differential diagnosis with NCL1. Beta-galactosidase (EC 3.2.1.23) was also measured as a sample quality control. Metabolomics has demonstrated that DBS are stable where collected and transported within 28 days at room temperature (21). All samples in this study were received within one week of collection.

We adapted the methods of van Diggelen et al. (22, 23) and Ho and O'Brien (24) to evaluate the enzymatic activity of PPT1, TPP1, and β-galactosidase, respectively. Briefly, for the TPP1 measurement in DBS, a 3.2-mm punch was incubated for 20 h at 37°C with 40 μL of substrate comprising Ala-Ala-Phe-7-amido-4-MC 0.5 mM and 20 μL of sodium chloride 0.85%. The reaction was stopped with 200 μL of ethylenediamine. Substrate was added to the blanks after addition of the stopping buffer. For the PPT1 measurement, a 3.2-mm punch was incubated for 5 h at 37°C with 40 μL of substrate 4-MU-6-thio-palmitate-β-D-glucopyranoside 0.64 mM. The reaction was stopped with 300 μL of ethylenediamine.

For the biochemical confirmatory test, leukocytes were isolated from blood in EDTA tubes using the Wizard® Genomic DNA Purification Kit (Promega) and stored at −20°C until use. The leukocyte samples were diluted in 0.9 % sodium chloride solution and sonicated in an Ultrasonic Sonicator Processor (Bandelin Sonopuls HD 2070). The Bradford method was used to quantify protein in leukocytes (25). For the TPP1 measurement, 10 μg of protein was incubated for 2 h at 37°C with 20 μL of substrate comprising Ala-Ala-Phe-7-amido-4-MC 0.5 mM and 40 μL of 0.425% sodium chloride solution. The reaction was stopped with 300 μL of ethylenediamine. In the case of PPT1, 15 μg of protein was incubated for 1 h

TABLE 2 | Clinical signs reported from patients with suspected NCL2.

Signs and symptoms	Number	%
Language delay	24	15.5
Psychomotor delay	21	13.5
Epilepsy (refractory)	21 (8)	13.5
Myoclonic epilepsy	9	5.8
Myoclonic seizure	8	5.2
Regression	8	5.2
Visual deficit	8	5.2
Gait alteration/absence	8	5.2
Ataxia	7	4.5
Seizures	6	3.9
Encephalopathy	6	3.9
Cerebellar atrophy	4	2.6
Microcephaly	4	2.6
Hypotonia	4	2.6
Spasticity	3	1.9
Cortical atrophy	2	1.3
Aggressiveness	2	1.3
Stereotypies	1	0.6

at 37°C with 20 μL of substrate 4-MU-6-thio-palmitate-β-D-glucopyranoside 0.64 mM. The reaction was stopped with 300 μL of ethylenediamine.

Fluorescence (excitation, 355 nm; emission, 460 nm) was measured on a BMG Labtech spectrofluorometer (FluoStar Optima). The readings were corrected for blanks and compared with 4-MU calibrators in the case of PPT1 and β-galactosidase, and with 7-amino-4-MC calibrators for TPP1. Enzyme activities were expressed in micromoles of 4-MU or 4-MC of product formed per h/L of blood (DBS samples) or nanomole per h/mg of protein (leukocytes) for PPT1 and TPP1, respectively.

RESULTS

A total of 143 kits were distributed between March 1, 2017 and May 1, 2020. Samples from 71 patients (age range: 2.5 months−15 years, 27 females, and 44 males) were received from 21 of the 17 regions of Spain. Madrid accounted for 24.4% and Valencia 13.4%. It is a minimally invasive test with a total cost per sample of around €50, compared to €80 to €100 for leukocytes and €300 for fibroblasts.

The main manifestations reported were language delay (15.5%), psychomotor delay (13.5%), and epilepsy (13.5%) (**Table 2**).

Analysis of the DBS enzyme enabled identification of three patients with absent activity in TPP1 but normal PPT1 activity (**Supplementary Table 1**; **Table 3**). In patient 3, we also confirmed leukocyte deficiency. All cases were confirmed by genetic analysis, and three known pathogenic variants were identified (**Table 3**); variant c.622C>T was present in all three patients, in one patient in homozygosity, and in two patients in compound heterozygosity with variants c.509-1G>C and c.1094G>A, respectively. We only had samples from both parents for patient 3 and were able to confirm the inheritance pattern in trans in the TPP1 gene. These three cases identified as a direct result of the selective screening strategy were counted as true positives. In all samples, DBS and/or leukocytes, determination of β-galactosidase revealed normal values (data not shown).

All patients had similar ages at diagnosis (**Table 3**), ranging from four to five years, and shared common features such as epileptic seizures and motor decline. Ataxia and language decline were also recorded in two patients.

Patient 1, a male whose symptoms first manifested at 12 months of age as language delay followed by full stagnation of development at 15 months and cognitive deterioration at four years of age, as reported by the parents. His motor development was reported as normal. He experienced his first seizure at 3.5 years, followed by refractory epilepsy at age five despite treatment with valproate, clobazam, and levetiracetam. He presented tonic, atonic, tonic-clonic, and myoclonic seizures, together with myoclonic and atonic absences. He showed myoclonus (3.5 years), spasticity, and dystonia (4–4.5 years) with motor regression and total loss of gait at the age of five. Visual impairment was observed at 3.5 years, progressing to total blindness at five. Intraventricular ERT was not started after the diagnosis, as the child did not meet the inclusion criteria because of the severity of his disease. He died at the age of 6 years old.

Patient 2, a female whose symptoms first manifested at 12 months of age as language delay followed by cognitive deterioration starting at 2.5 years of age and coinciding with the first seizures. She experienced absences and right focal tonic-clonic seizures, requiring three antiepileptic drugs due to refractory epilepsy. She also had axial tremor, myoclonus, oro-facial dyskinesias, and oculomotor apraxia. No visual deficit was recorded. ERT was initiated at the age of diagnosis (five years and six months), although it was interrupted after six months due to lack of response and deterioration.

Patient 3, a male who showed the first symptoms of the disease at 3.5 years of age, namely, mild language delay and clumsiness. Cognitive decline was observed at four years of age, coinciding with the onset of epilepsy. He experienced his first seizure at three years and 11 months of age, followed by refractory epilepsy at age five. He currently requires treatment with three anticonvulsants: valproate, clobazam, and levetiracetam. His seizures are tonic, atonic, tonic-clonic, and myoclonic, together with absences. He also has myoclonus and spasticity, together with ataxia, tremor, and choreic movements of the upper extremities. He has needed a wheelchair since the age of five years, although he is able to crawl. Visual deficit has not been evidenced. Intraventricular ERT was started at the age of diagnosis (five years). He remains stable after one year on therapy. Currently, at six years of age he has a comprehensive language, and although expressive language is mostly unintelligible, he tries to communicate and says some words with meaning.

DISCUSSION

Clinician awareness and regional differences in availability of TPP1 testing may affect prompt diagnosis. Furthermore, ensuring that clinicians are aware of NCL2 during the differential diagnosis may facilitate early treatment with ERT. LINCE is

TABLE 3 | Characterization of CLN2 patients diagnosed in the LINCE project.

	Age at diagnosis	TPP1 DBS (μmol/L/h) (2.0–11.0)	PPT1 DBS (μmol/L/h) (5.4–40.1)	TPP1 leukocytes (nmol/h/mg) (24–68)	PPT1 leukocytes (nmol/h/mg) (1.9–25.4)	CLN2 Genotype	Clinical signs reported at diagnosis
Patient 1	4 years 6 months	Undetectable	9.1	Nd	Nd	c.622C>T/ c.622C>T	Delayed speech and language development Mental deterioration and motor deterioration Cerebellar atrophy by MRI Ataxia Epilepsy
Patient 2	5 years 6 months	Undetectable	13.2	Nd	Nd	c.509-1G>C/c.622C>T	Loss of speech Loss of gait Epileptic crisis
Patient 3	5 years	Undetectable	8.8	Undetectable	27.8	c.622C>T/c.1094G>A	Cognitive and motor decline Cerebellar atrophy by MRI Ataxia Epilepsy

an acronym for Spanish Neuronal Ceroidea Lipofuscinosis. Our goal is not only to speed up the diagnosis of NCL2 disease for physicians who suspect a NCL, but also to create awareness of these dramatic, debilitating diseases. In this study, we demonstrated that diagnosis of NCL2 is possible using a simple, selective screening method. The use of DBS as a first-tier sample is very convenient, since it is easy to collect and remains stable at room temperature; therefore, it can be sent by ordinary mail (26–28). It is also very cost-effective compared with leukocyte or fibroblast samples (13, 29), which can be particularly sensitive to temperature variations and shipping delays, thus ultimately reducing assay reliability (13). The diagnostic method is based on fluorometric enzyme analysis for both DBS and leukocyte samples, and no residual activity was found in any sample from NCL2 patients. No false-negative results have been reported to date, and no NCL1 patients have been identified. More recently, tandem mass spectrometry (MSMS) assays for the determination of TPP1, PPT1 and cathepsin D activity in DBS have become available (30–34). Although MSMS assays yield higher analytical ranges than fluorometric assays (34), one of the advantages of our method is that it can measure PPT1 in only 5 h and TPP1 in 20 h, as opposed to the 45-h incubation reported by Lukacs et al. (26) and similar to LC-MS/MS multiplex assays (34). Efforts are currently being made in our laboratory to measure both TPP1 and PPT1 in the shortest time possible using mass spectrometry, because are the most commons.

Unfortunately, since NCL2 and NCL1 are among the few NCLs with lysosomal soluble proteins that can be measured to support a diagnosis, genetic analysis is the only diagnostic option for the remaining NCLs. Two of the patients in the present series were compound heterozygous, the first was homozygous, and the variants had already been described and classified as pathogenic (35). Of the 131 known NCL2 variants (34), c.509-1G>C and c.622C>T were found in all three patients. These variants are the two most frequent and account for 50% of disease-associated alleles (89% in Europe). Both variants have been associated with classic late-infantile and atypical NCL2 (8% juvenile, 3% spinocerebellar ataxia, and <1% spastic paraplegia or congenital disease), suggesting that additional genetic factors are likely to contribute to earlier disease onset. The other variant, c.1094G>A, represents 2% of disease associated alleles and may be associated with an increased probability of classic late-infantile NCL2. However, specific disease genotypes do not consistently correlate with phenotypes (36).

Several therapeutic approaches for NCLs are currently under investigation, including gene therapy, neuronal stem cell therapy, immunomodulation, small molecule therapy, and ERT (37–41), which is the only approved treatment for NCL2. Cerliponase alfa, a recombinant proenzyme form of human TPP1, was granted regulatory approval in the USA and Europe for the treatment of NCL2 disease (specifically for slowing the loss of ambulation in symptomatic children aged ≥3 years in the USA). It is administered by intraventricular infusion every 2 weeks and has demonstrated less decline in motor and language function than in historical controls. The maximum benefit from ERT can be obtained if diagnosis is made as early as possible in order to avoid the irreversible neurodegeneration often present when diagnosis is made at around five years of age, and also because the disease progresses very rapidly immediately after onset of the initial symptoms. As can be seen from the most common signs (**Table 2**), language delay and/or psychomotor delay are often associated with unexplained seizures, which should raise the suspicion of NCL2.

Limitations

The study has been carried out in patients with symptoms and with voluntary participation, because of this does not reflect the real incidence.

CONCLUSIONS

In summary, the LINCE project is a simple, useful, not very expensive, and novel tool that speeds up the diagnosis of NCL2. All positive cases experienced a rapid decline in their medical condition, which is characteristic of NCL2 patients. An early diagnosis is key to accessing treatment, but diagnosis is challenging because of similarities with other disorders and limited awareness of NCL2 disease because of its rarity. Since therapy is now available, screening for NCL2 should be implemented in neonatal screening programs for early detection, ultimately leading to better outcomes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee Santiago-Lugo with the register number 2017/185. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

CC, MJC, and MLC contributed to design of study. DR, PC, and CC contributed to analysis and interpretation of data. AD-R, AM, and MT contributed to acquisition of clinical data. All authors have participated and/or reviewing, the manuscript and have approved its submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.876688/full#supplementary-material

REFERENCES

1. Pierret C, Morrison JA, Kirk M. Treatment of lysosomal storage disorders: focus on the neuronal ceroid-lipofuscinoses. *Acta Neurobiol Exp (Wars).* (2008) 68:429–42.
2. Sleat DE, Gedvilaite E, Zhang Y, Lobel P, Xing J. Analysis of large-scale whole exome sequencing data to determine the prevalence of genetically-distinct forms of neuronal ceroid lipofuscinosis. *Gene.* (2016) 593:284–91. doi: 10.1016/j.gene.2016.08.031
3. Nita DA, Mole SE, Minassian BA. Neuronal ceroid lipofuscinoses. *Epileptic Disord.* (2016) 18:73–88. doi: 10.1684/epd.2016.0844
4. Kohlschütter A, Schulz A. CLN2 disease (Classic late infantile neuronal ceroid lipofuscinosis). *Pediatr Endocrinol Rev.* (2016) 13:682–8.
5. Beltrán L, Valenzuela GR, Loos M, Vargas R, Lizama R, Spinsanti P, et al. Late–onset childhood neuronal ceroid lipofuscinosis: early clinical and electroencephalographic markers. *Epilepsy Res.* (2018) 144:49–52. doi: 10.1016/j.eplepsyres.2018.05.005
6. Rawlings ND, Barret AJ. Tripeptidyl–peptidase I is apparently the CLN2 protein absent in classical late–infantile neuronal ceroid lipofuscinosis. *Biochim Biophys Acta.* (1999) 1429:496–500. doi: 10.1016/S0167-4838(98)00238-6
7. Vines DJ, Warburton MJ. Classical, late infantile neuronal ceroid lipofuscinosis fibroblasts are deficient in lysosomal tripeptidyl peptidase I. *FEBS Lett.* (1999) 443:131–5. doi: 10.1016/S0014-5793(98)01683-4
8. Warburton MJ, Bernardini F. Tripeptidyl–peptidase I deficiency in classical late–infantile neuronal ceroid lipofuscinosis brain tissue. Evidence for defective peptidase rather than proteinase activity. *J Inherit Metab Dis.* (2000) 23:145–54. doi: 10.1023/A:1005665732189
9. Sleat DE, Donelly RJ, Lackland H, Liu CG, Sohar I, Pullarkat RK, et al. Association of mutations in a lysosomal protein with classical late–infantile neuronal ceroid lipofuscinosis. *Science.* (1997) 277:1802–5. doi: 10.1126/science.277.5333.1802
10. Kyttälä A, Lahtinen U, Braulke T, Hoffmann SL. Functional biology of the neuronal ceroid lipofuscinosis (NCL) proteins. *Biochim Biophys Acta.* (2006) 1762:920–33. doi: 10.1016/j.bbadis.2006.05.007
11. Haltia M. The neuronal ceroid–lipofuscinoses: from past to present. *Biochim Biophys Acta.* (2006) 1762:850–6. doi: 10.1016/j.bbadis.2006.06.010
12. Radke J, Stenzel W, Goebel HH. Human NCL neuropathology. *Biochim Biophys Acta.* (2015) 1852:2262–6. doi: 10.1016/j.bbadis.2015.05.007
13. Fietz M, AlSayed M, Burke D, Cohen-Pfeffer J, Cooper JD, Dvoráková L, et al. Diagnosis of neuronal ceroid lipofuscinosis type 2 (CLN2 disease): expert recommendations for early detection and laboratory diagnosis. *Mol Genet Metab.* (2016) 119:160–7. doi: 10.1016/j.ymgme.2016.07.011
14. Dozières-Puyravel B, Nasser H, Elmaleh-Bergès M, Lopez Hernandez E, Gelot A, Ilea A, et al. Paediatric–onset neuronal ceroid lipofuscinosis: first symptoms and presentation at diagnosis. *Dev Med Child Neurol.* (2020) 62:528–30. doi: 10.1111/dmcn.14346
15. Williams RE, Adams HR, Blohm M, Cohen-Pfeffer JL, de Los Reyes E, et al. Management strategies for CLN2 disease. *Pediatr Neurol.* (2017) 69:102–12. doi: 10.1016/j.pediatrneurol.2017.01.034
16. Nickel M, Simonati A, Jacoby D, Lezius S, Kilian D, Van de Graaf B, et al. Disease characteristics and progression in patients with late–infantile neuronal ceroid lipofuscinosis type 2 (CLN2) disease: an observational cohort study. *Lancet Child Adolesc Health.* (2018) 2:582–90. doi: 10.1016/S2352-4642(18)30179-2
17. Markham A. Cerliponase alfa: first global approval. *Drugs.* (2017) 77:1247–9. doi: 10.1007/s40265-017-0771-8
18. Specchio N, Pietrafusa N, Trivisano M. Changing times for CLN2 disease: the era of enzyme replacement therapy. *Ther Clin Risk Manag.* (2020) 16:213–22. doi: 10.2147/TCRM.S241048
19. Lewis G, Morrill AM, Conway-Allen SL, Kim B. Review of cerliponase alfa: recombinant human enzyme replacement therapy for late–infantile neuronal ceroid lipofuscinosis type 2. *J Child Neuro.* (2020) 35:348–53. doi: 10.1177/0883073819895694

20. Schulz A, Ajayi T, Specchio N, de Los Reyes E, Gissen P, Ballon D, et al. Study of intraventricular cerliponase alfa for CLN2 disease. *N Engl J Med.* (2018) 378:1898–907. doi: 10.1056/NEJMoa1712649
21. Palmer EA, Cooper HJ, Dunn WB. Investigation of the 12-month stability of dried blood and urine spots applying untargeted UHPLC-MS metabolomic assays. *Anal Chem.* (2019) 91:14306–13. doi: 10.1021/acs.analchem.9b02577
22. van Diggelen OP, Keulemans JLM, Winchester B, Hofman IL, Vanhanen SL, Santavuori P, et al. A rapid fluorogenic Palmitoyl–protein thioesterase assay: pre- and postnatal diagnosis of NCL. *Mol Genet Metab.* (1999) 66:240–4. doi: 10.1006/mgme.1999.2809
23. van Diggelen OP, Keulemans JLM, Kleijer WJ, Thobois S, Tilikete C, Voznyi YV. Pre- and postnatal enzyme analysis for infantile, late infantile and adult neuronal ceroid lipofuscinosis (CLN1 and CLN2). *Eur J Paediatr Neurol.* (2001) 5(Suppl. A):189–92. doi: 10.1053/ejpn.2000.0460
24. Ho MW, O'Brien JS. Differential effect of chloride ions on β-galactosidase isoenzymes: a method for separate assay. *Clin Chim Acta.* (1971) 32:443–50. doi: 10.1016/0009-8981(71)90446-3
25. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal Biochem.* (1976) 72:248–54. doi: 10.1016/0003-2697(76)90527-3
26. Lukacs Z, Santavuori P, Keil A, Steinfeld R, Kohlschütter A. Rapid and simple assay for the determination of tripeptidyl peptidase and palmitoyl protein thioesterase activities in dried blood spots. *Clin Chem.* (2003) 49:509–11. doi: 10.1373/49.3.509
27. Itagaki R, Endo M, Yanagisawa H, Hossain MA, Akiyama K, Yaginuma K, et al. Characteristics of PPT1 and TPP1 enzymes in neuronal ceroid lipofuscinosis (NCL) 1 and 2 by dried blood spots (DBS) and leukocytes and their application to newborn screening. *Mol Genet Metab.* (2018) 124:64–70. doi: 10.1016/j.ymgme.2018.03.007
28. Lukacs Z, Nickel M, Murko S, Nieves Cobos P, Schulz A, Santer R, et al. Validity of a rapid and simple fluorometric tripeptidyl peptidase 1 (TPP1) assay using dried blood specimens to diagnose CLN2 disease. *Clin Chim Acta.* (2019) 492:69–71. doi: 10.1016/j.cca.2019.02.010
29. Young EP, Worthington VC, Jackson M, Winchester BG. Pre- and postnatal diagnosis of patients with CLN1 and CLN2 by assay of palmitoyl-protein thioesterase and tripeptidyl-peptidase I activities. *Eur J Paediatr Neurol.* (2001) 5(Suppl. A):193–6. doi: 10.1053/ejpn.2000.0461
30. Barcenas M, Xue C, Marushchak–Vlaskin T, Scott CR, Gelb MH, Tureček F. Tandem mass spectrometry assays of palmitoyl protein thioesterase 1 and tripeptidyl peptidase activity in dried blood spots for the detection of neuronal ceroid lipofuscinoses in newborns. *Anal Chem.* (2014) 86:7962–8. doi: 10.1021/ac501994b
31. Liu Y, Yi F, Kumar AB, Kumar Chennamaneni N, Hong X, Scott CR et al. Multiplex tandem mass spectrometry enzymatic activity assay for newborn screening of the mucopolysaccharidoses and type 2 neuronal ceroid lipofuscinosis. *Clin Chem.* (2017) 63:1118–26. doi: 10.1373/clinchem.2016.269167
32. Khaledi H, Liu Y, Masi S, Gelb MH. Detection of infantile Batten disease by tandem mass spectrometry assay of PPT1 enzyme activity in dried blood spots. *Anal Chem.* (2018) 90:12168–71. doi: 10.1021/acs.analchem.8b03188
33. Kumar AB, Masi S, Ghomashchi F, Chennamaneni NK, Ito M, Scott CR, et al. Tandem mass spectrometry has a larger analytical range than fluorescence assays of lysosomal enzymes: application to newborn screening of mucopolysaccharidoses types II, IVA and VII. *Clin Chem.* (2015) 61:1363–71. doi: 10.1373/clinchem.2015.242560
34. Maeser S, Petre BA, Ion L, Rawer S, Kohlschütter A, Santorelli FM, et al. Enzymatic diagnosis of neuronal lipofuscinoses in dried blood spots using substrates for concomitant tandem mass spectrometry and fluorimetry. *J Mass Spectrom.* (2021) 56:e4675. doi: 10.1002/jms.4675
35. Gardner E, Bailey M, Schulz A, Aristorena M, Miller N, Mole SE. Mutation update: review of TPP1 gene variants associated with neuronal ceroid lipofuscinosis CLN2 disease. *Hum Mutat.* (2019) 40:1924–38. doi: 10.1002/humu.23860
36. Mole SE. The genetic spectrum of human neuronal ceroid–lipofuscinoses. *Brain Pathol.* (2004) 14:70–6. doi: 10.1111/j.1750-3639.2004.tb00500.x
37. Kohlschütter A, Schulz A, Bartsch U, Storch S. Current and emerging treatment strategies for neuronal ceroid lipofuscinoses. *CNS Drugs.* (2019) 33:315–25. doi: 10.1007/s40263-019-00620-8
38. Johnson TB, Cain JT, White KA, Ramirez-Montealegre D, Pearce DA, Weimer JM. Therapeutic landscape for Batten disease: current treatments and future prospects. *Nat Rev Neurol.* (2019) 15:161–78. doi: 10.1038/s41582-019-0138-8
39. Mole SE, Anderson G, Band HA, Berkovic SF, Cooper JD, Kleine Holthaus SM, et al. Clinical challenges and future therapeutic approaches for neuronal ceroid lipofuscinosis. *Lancet Neurol.* (2019) 18:107–16. doi: 10.1016/S1474-4422(18)30368-5
40. Haney MJ, Zhao Y, Jin YS, Batrakova EV. Extracellular vesicles as drug carriers for enzyme replacement therapy to treat CLN2 Batten Disease: optimization of drug administration routes. *Cells.* (2020) 9:1273. doi: 10.3390/cells9051273
41. Mazurkiewicz-Bełdzińska M, Del Toro M, Haliloglu G, Huidekoper HH, Kravljanac R, Mühlhausen C, et al. Managing CLN2 disease: a treatable neurodegenerative condition among other treatable early childhood epilepsies. *Expert Rev Neurother.* (2021) 21:1275–82. doi: 10.1080/14737175.2021.1885374

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REVIEW
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Glial Dysfunction and Its Contribution to the Pathogenesis of the Neuronal Ceroid Lipofuscinoses

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While significant efforts have been made in developing pre-clinical treatments for the neuronal ceroid lipofuscinoses (NCLs), many challenges still remain to bring children with NCLs a cure. Devising effective therapeutic strategies for the NCLs will require a better understanding of pathophysiology, but little is known about the mechanisms by which loss of lysosomal proteins causes such devastating neurodegeneration. Research into glial cells including astrocytes, microglia, and oligodendrocytes have revealed many of their critical functions in brain homeostasis and potential contributions to neurodegenerative diseases. Genetically modified mouse models have served as a useful platform to define the disease progression in the central nervous system across NCL subtypes, revealing a wide range of glial responses to disease. The emerging evidence of glial dysfunction questions the traditional "neuron-centric" view of NCLs, and would suggest that directly targeting glia in addition to neurons could lead to better therapeutic outcomes. This review summarizes the most up-to-date understanding of glial pathologies and their contribution to the pathogenesis of NCLs, and highlights some of the associated challenges that require further research.

Keywords: Batten disease, neuronal ceroid lipofuscinosis, astrocyte, microglia, oligodendrocyte

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INTRODUCTION

Lysosomal storage disorders (LSDs) are a group of more than 70 monogenetic diseases characterized by defects in lysosomal metabolism and subsequent accumulation of substrates. Most LSDs present with a broad phenotypic spectrum in multiple organs. This is consistent with the fact that nearly all lysosomal enzymes are ubiquitously expressed and their deficiency will therefore affect many tissue types (1). The neuronal ceroid lipofuscinoses (NCLs or Batten disease) are a group of fatal neurodegenerative LSDs affecting children and young adults. In contrast to other non-neuronopathic LSDs, the NCLs primarily affect the central nervous system (CNS), usually including the retina. The NCLs are remarkably heterogeneous diseases, with studies in both humans and animal models showing that each of 13 subtypes is caused by mutations in different individual genes and have different ages of onset, clinical symptoms, and rate of disease progression (2, 3) (**Table 1**). As comprehensively reviewed elsewhere (2, 3), a mutation (or mutations) in a different NCL gene causes each form of NCL. Some of these mutations are in soluble lysosomal enzymes

(e.g., CLN1, CLN2, CLN10, CLN13), others are in transmembrane proteins within the lysosome (e.g., CLN3, CLN7) or elsewhere in the cell (e.g., CLN6, CLN8), or a range of proteins that vary widely in their nature and location (e.g., CLN4, CLN5, CLN11, CLN12, CLN14).

Research into treatments for most LSDs has primarily focused on the replacement of the missing gene product responsible for each disease. Enzyme replacement therapy (ERT) for several soluble enzyme-deficient forms of NCL including CLN1 and CLN2 diseases has been studied (11, 34–38), which led to the recent FDA approval of cerliponase alfa for CLN2 disease (39). However, ERT is only disease-modifying, and several longer-term challenges regarding whether efficacy will be maintained, the delivery systems used and potential immune responses remain (36, 37, 40). Furthermore, ERT is not an option for those subtypes of NCL caused by defects in transmembrane proteins such as CLN3 disease, which is the most common form of NCL (2). Viral vector-mediated gene therapy has been intensively explored as an alternative therapeutic strategy for the NCLs. This approach theoretically has the advantage that a single one-time administration of viral vector should restore deficient lysosomal proteins to transduced cells (41, 42). Preclinical studies of gene therapy in animal models of CLN1, CLN2, CLN3, CLN6, CLN7, and CLN10 diseases have shown promising results (4, 23, 34, 43–47). However, clinical studies in children with CLN2 disease treated with gene therapy showed considerably less efficacy (48, 49), highlighting the difficulty of translating advances from mice directly into human patients (50). Indeed, none of the therapies that are currently available or being tested clinically are curative. Therefore, devising optimal therapeutic strategies for the NCLs will certainly require a better understanding of pathophysiology in each form of NCL.

Neuropathology in the NCLs was initially characterized in human autopsy studies, revealing marked neuron loss accompanied by intra-lysosomal accumulation of autofluorescent storage material (AFSM), whose major protein component is subunit C of mitochondrial ATP synthase (SCMAS), in addition to astrogliosis, and microglial activation (51, 52). With the limited availability of genetically validated human autopsy samples, many longitudinal studies in animal models have been performed, in order to understand the staging of neuropathological processes from the earliest events to the end-stage of disease. Interestingly, AFSM accumulation, astrogliosis, microglial activation and neuron loss in animal models of NCL are remarkably selective in their early stages, becoming more widespread with disease progression (53). This suggests that despite the ubiquitous expression of these proteins, such selective vulnerability may be due to them playing physiological roles of greater importance in some cell populations than others.

A significant finding made in multiple mouse models across subtypes of NCL is the profound loss of thalamic neurons, which typically precedes neuron loss in the corresponding region of the cortex to which these thalamic neurons relay (5, 14, 16, 17, 20, 28, 30, 54). Strikingly, these studies in mouse models also revealed that localized astrocytic and microglial activation, which both occur early in disease progression, accurately predict where subsequent selective neuron loss occurs in mouse models of a majority of NCL subtypes. Such findings cast doubt on traditional perspectives of the NCLs as predominantly "neuronal" diseases, and lead to the hypothesis that abnormalities in glial cells may contribute to the neurodegeneration associated with the NCLs.

In the "neuron-centric" past of neuroscience, glial cells were often relegated to being considered as undefined passive structural elements, and in the diseased state glial activation was often considered a secondary response to neuron dysfunction or damage. Over recent decades, this traditional neuron-centric conception of the CNS has been challenged by a large body of research aiming to provide a better understanding of glial function, revealing that glial cells including astrocytes, microglia, and oligodendrocytes have more active roles in both neuronal homeostasis and neurodegeneration (55–57). Notably, recent technological advancements have enabled us to study the heterogeneity of each glial cell type, and have revealed their bimodal or multimodal roles in neurodegenerative diseases (58, 59). This review aims to summarize the recent progress in our understanding of glial pathologies and their contribution to NCL pathogenesis and examines where NCL research currently stands in the field of glial biology. This review focusses primarily upon CLN1, CLN2 and CLN3 diseases as the three most common forms of NCL, in which a consideration of glial dysfunction or the contribution to pathogenesis has been undertaken or is underway. However, where available, the extent of astrogliosis and microglial activation or oligodendrocyte pathology is listed in mouse models of other forms of NCL in (**Table 1**). As discussed below, these immunohistochemically detectable changes may be due to dysfunction of glial cell types (which is largely unexplored in most NCLs), or reflect their response to ongoing neuronal dysfunction or loss.

GLIAL DYSFUNCTION IN THE NCLs

Astrocytes

Neuroimmune responses mediated by both astrocytes and microglia have crucial roles in all CNS insults including brain injury, infection, and neurodegenerative diseases (60, 61). In response to these insults, astrocytes and microglia become "activated" or "reactive" by altering their morphology, protein expression, and secretion profile. The fact that astrocytes and microglia typically both become activated in concert has made it difficult to distinguish the relative contributions of astrocytes to neurodegeneration, and whether these are distinct from those of microglia. Nonetheless, understanding their distinct patterns of activation in disease states is very important.

Upregulation of intermediate filaments, most notably glial fibrillary acidic protein (GFAP), is a classic marker for astrogliosis in mammalian models, and the expression level of GFAP or immunohistochemical detection of this marker has proved a useful tool to assess the extent of astrogliosis (62). As summarized in **Table 1**, GFAP-positive astrogliosis has been documented in all characterized mouse models of NCL. Although astrogliosis is observed in multiple CNS regions toward the end stage of disease, typical astrogliosis in the NCLs is characterized by its regional specificity and timing; astrogliosis especially occurs

TABLE 1 | Summary of glial changes in mouse models of neuronal ceroids lipofuscinoses.

Subtype	Gene	Mouse model	Astrocyte activation	Microglial activation	Oligodendrocytic pathology	References
CLN1	*CLN1/PPT1*	*Ppt1*$^{-/-}$	GFAP+ astrogliosis within spinal cords at 2 months, M1, S1BF, VPM/VPL, LGNd, MGN, CM, and Rt at 3 months, and hippocampus at 7 months.	CD68+ activation within spinal cords at 1 months, F4/80+ activation within M1, S1BF, V1, VPM/VPL, LGNd, and MGN at 5 months and hippocampus at 7 months	Decreased white matter volume in spinal cords at 2–3 months; increased immunoreactivity in Olig2, NG2, and MBP within spinal cords at 1–2 months	(4), (5) (6), (7)
		Ppt1$^{\Delta ex4}$	GFAP+ astrogliosis within cortex at 3 months	F80+ activation within thalamus at 3 months	N/A	(8)
		Ppt1^{R151X}	GFAP+ astrogliosis within cortex	CD68+ activation within cortex	N/A	(9)
		Cln1^{R151X}	GFAP+ astrogliosis within cortex, thalamus, and hippocampus	CD68+ activation within cortex, thalamus, and hippocampus	N/A	(10)
CLN2	*CLN2/ TPP1*	*Tpp1*$^{-/-}$	GFAP+ astrogliosis within M1 at 2 months and striatum and hippocampus at 3 months	Increase in Iba1 immunoreactivity whithin striatum at 3 months	N/A	(11), (12)
		Cln2^{R207X}	GFAP+ astrogliosis within cortex at 3 months	No change in Iba1 immunoreactivity at 3 months	N/A	(13)
CLN3	*CLN3*	*Cln3*$^{\Delta ex1-6}$	GFAP+ astrogliosis whithin visual cortex, hippocampus, striatum, and cerebellum at 5 months and somatosensory cortex at 7 months	F4/80+ activation within cortex, hippocampus, striatum, and cerebellum at 5 months	N/A	(14), (15)
		Cln3$^{\Delta ex7-8}$	GFAP+ astrogliosis within cortex, striatum, VPM/VPL, and cerebellum at 12 months	F4/80+ astivation within cortex, striatum, VPM/VPL, and cerebellum at 12 months	N/A	(16), (17)
		Cln3^{Q352X}	GFAP+ astrogliosis within S1BF and VPM/VPL at 6 months	CD68+ activation within S1BF and VPM/VPL at 6 months	N/A	(18)
CLN4	*CLN4/ DNAJC5/CSP*	*Csp*$^{-/-}$	N/A mice die at 2–4 weeks old	N/A mice die at 2–4 weeks old	N/A mice die at 2–4 weeks old	(19)
CLN5	*CLN5*	*Cln5*$^{-/-}$	GFAP+ astrogliosis within S1BF, V1, and VPM/VPL at 1 months and LGNd at 12 months	F4/80+ activation within S1BF, V1, VPM/VPL, and LGNd at 12 months	Reduced MBP+ fibers in S1BF at 1–3 months	(20), (21)
		Cln5$^{\Delta ex3}$	Upregulation of GFAP mRNA in cerebrams at 4.5 months	N/A	Downregulation of MBP and MOG mRNA at 3 months, MAG and PLP mRNA at 4.5 months	(22)
CLN6	*CLN6*	*Cln6*^{nclf}	GFAP+ astrogliosis within V1, LGNd, and SC at 12 weeks, VPM/VPL and striatum at 21 weeks, and cerebellum at 54 weeks	CD68+ activation within V1, LGNd, and SC at 12 weeks, VPL/VPM, hippocampus, and cerebellum at 54 weeks	N/A	(23), (24)
CLN7	*CLN7/MFSD8*	*Mfsd8*$^{tm1a/tm1a}$	GFAP+ astrogliosis within cerebellar white matter at 10 months	CD68+ activation within cerebellum, spinal cord and thalamus at 10 months	N/A	(25)
		Cln7$^{-/-}$	GFAP+ astrogliosis within cortex, hippocampus, thalamus, medulla, erebellum, and spinal cord at 5 months	CD68+ activation within cortex, hippocampus, thalamus, medulla, and cerebellum at 7 months	N/A	(26), (27)
CLN8	*CLN8*	*Cln8*^{mnd}	GFAP+ astrogliosis within VPM/VPL, S1BF, and V1 at 5 months and within LGNd at 8 months	CD68+ activation whithin VPM/VPL, S1BF, V1, and LGNd at 5 months	Decreased white matter volume in corpus callosum and internal capsule at 1–3 months; decreased expression level of MBP and PLP at 1 month; increased G-ration in corpus callosum at 1–4 months	(28), (29)
CLN10	*CTSD*	*Ctsd*$^{-/-}$	Widespread GFAP+ astrogliosis, particularly prominent whithin thalamus and cortex laminae IV-VI at 24 days	CD68+ activation whithin thalamus and substantia nigra at 24 days	N/A	(30)

(Continued)

TABLE 1 | Continued

Subtype	Gene	Mouse model	Astrocyte activation	Microglial activation	Oligodendrocytic pathology	References
CLN11	*GRN*	$Grn^{-/-}$	GFAP+ astrogliosis within hippocampus, cortex, and thalamus at 24 months	Increased Iba-1 immunoreactivity within hippocampus, cortex, and thalamus at 24 months	N/A	(31)
CLN12	*ATP13A2*	$Atp13a2^{-/-}$	GFAP+ astrogliosis within cortex at 1 month, cerebellum, hippocampus, and midbrain at 18 months	N/A	N/A	(32)
CLN13	*CTSF*	$Ctsf^{-/-}$	GFAP+ astrogliosis in thalamocortical system at 12 months	F4/80+ microglial activation in thalamocortical system at 12 months	N/A	(33)
CLN14	*KCTD7*	N/A	N/A	N/A	N/A	N/A
CLN15 (proposed)	*TBCK*	N/A	N/A	N/A	N/A	N/A

M1, primary motor cortex; S1BF, somatosensory cortex barrel field; V1, primary visual cortex; VPM/VPL, medial and lateral ventral posterior nuclei; LGNd, dorsal lateral geniculate nucleus; MGN, medical geniculate; MD, mediodorsal nucleus; CM, central medial thalamic nucleus; Rt, reticular nucleus of thalamus; SC, superior colliculus; GFAP, glial fibrillary associated protein; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; MAG, myelin-associate glycoprotein; PLP, proteolipid protein.

early and is pronounced in thalamocortical pathways where considerable subsequent neuron loss occurs (reviewed in 26, see individual references in **Table 1**). This is in contrast to many other neuropathic LSDs such as mucopolysaccharidosis (MPS), in which astrogliosis tends to be more generalized across the CNS throughout disease progression (63, 64). Interestingly, the extent of GFAP reactivity and morphological alteration in astrocytes varies across the NCLs. For example, hypertrophy of astrocyte cell bodies and processes and GFAP upregulation in $Cln3^{-/-}$ mice appears to be more subtle or perhaps attenuated compared to astrocytes observed in $Ppt1^{-/-}$ mice (14), implying that CLN1 and CLN3 diseases differ in the extent to which astrocytes are intrinsically dysfunctional and/or respond to extracellular stimuli. These differences in astrogliosis in CLN1 and CLN3 diseases are also recapitulated by *in vitro* experiments using primary astrocytes derived from the relevant mouse models; $Ppt1^{-/-}$ astrocytes exhibit a more activated morphology and higher expression levels of GFAP, and enhanced secretion of cytokine and chemokine compared with the wild-type astrocytes (65). In contrast, $Cln3^{-/-}$ astrocytes showed attenuated changes in morphology and GFAP expression in response to pharmacological stimulation with reduced secretion of a range of neuroprotective factors, mitogens, cytokines, and chemokines (66). It will therefore be important to further investigate the nature of astrocytic dysfunction using similar tissue culture methods for other forms of NCL such as CLN2 disease.

Recently, it has been demonstrated that GFAP depalmitoylation is regulated by PPT1, and blocking palmitoylation by the unique palmitoylated residue in GFAP attenuates both astrogliosis and the concurrent neurodegenerative pathology in CLN1 mice (67). This is the first evidence suggesting that loss of NCL proteins in astrocytes directly impacts an intrinsic astrocyte response rather than "reactive astrogliosis" occurring solely in response to ongoing neuronal damage. However, these findings appear somewhat contradictory to previous evidence showing that prevention of GFAP upregulation by knocking out both GFAP and Vimentin in $Ppt1^{-/-}$ mice ($Gfap^{-/-}$; $Vimentin^{-/-}$; $Ppt1^{-/-}$) exacerbates disease pathology, which had been interpreted as evidence for a protective role of GFAP upregulation in CLN1 disease (68). Not only do such findings imply multi-dimensional roles of astrogliosis, which will be discussed shortly, but also potentially different pathological impacts depending on NCL subtype, affected brain regions and staging of disease progression.

Recent efforts have focused on gene expression profiling of activated astrocytes both *in vitro* and *in vivo* to decipher their functional properties in the context of neurodegeneration. The paradigm of neurotoxic "A1" astrocytes and neuroprotective "A2" astrocytes is now a generally recognized concept (62, 69). Astrocytes resembling "A1" or neurotoxic status have been reported in more common neurodegenerative diseases such as Alzheimer's disease (AD) (70), amyotrophic lateral sclerosis (ALS) (71), and Parkinson's disease (72). Similarly, the pronounced typical A1-specific molecular signature has been recently reported in the forebrains of $Ppt1^{-/-}$ mice (73), suggesting a neurotoxic function of astrocytes in CLN1 disease. However, caution is needed in using the current A1/A2 classifications to interpret pathological roles of astrocytes, because such a binary A1/A2 paradigm may be an oversimplification of potentially more wide-ranging and heterogeneous states of astrogliosis (74). Indeed, the recent RNA sequencing data of $Tpp1^{-/-}$ mice have shown changes in the expression of a restricted subset of A1- or A2-specific genes, which does not match the typical A1/A2 classification (75). A lack of clear A1/A2 signature has also been reported in other diseases including Huntington disease (76), highlighting that astrocyte heterogeneity may convolute A1/A2 boundaries. Nevertheless, there is a potential that these widely accepted A1/A2 markers can still be useful for both investigating the pathological contribution of astrogliosis, comparing astrocyte phenotypes in the NCLs to other neurodegenerative conditions and assessing the efficacy of therapeutic approaches for NCLs.

Astrocytes also exert pathological influences on neuronal health through multiple non-inflammatory functions such as neurotransmitter recycling, ion buffering, and the release of growth factors (77, 78). In addition, the role of phagocytosis by astrocytes in synaptic connectivity is now in the spotlight but has been relatively understudied in neurodegenerative diseases (79). Considering their close relationship with lysosomal calcium signaling and lysosomal exocytosis, it is plausibly speculated that the loss of NCL proteins could affect many of these non-inflammatory functions of astrocytes. Impaired calcium signaling in primary astrocytes derived from *Ppt1*$^{-/-}$ and *Cln3*$^{-/-}$ mice has been documented (65, 66). Therefore, it will be important to decipher the molecular bases of possibly more diverse forms of astrocytic dysfunction in the NCLs rather than solely focusing on astrogliosis to better understand the pathological role of astrocytes in NCL pathogenesis.

Microglia

Microglia, the CNS tissue resident macrophage population, also become "activated" or "reactive" by changing their gene expression, morphology, motility, migration, metabolism, secretome, phagocytosis, proliferation, and death in response to CNS pathology (61). Microglial-astrocyte crosstalk via the release of diverse signaling molecules is particularly thought to mediate neurodegeneration (80), with recent studies suggesting that neurotoxic A1 astrocytes are triggered by fragmented mitochondria released from microglia to propagate and trigger neuronal death (81, 82).

Classically, immunoreactivity of several molecular markers including CD68, MHC antigen class II, F4/80, and Iba1 have been widely used to define the activated state of microglia (83, 84). Longitudinal studies using several of these markers have confirmed that where examined microglial activation is invariably present in the CNS of NCL mouse models, and anatomical distribution and onset of microglial activation largely overlap those of astrogliosis (**Table 1**). Although comprehensive profiling of multiple microglial markers is still underway, data so far suggest that the nature of microglial activation appears to be different in each NCL. This subtype-dependent nature of microglial activation is buttressed by *in vitro* primary culture experiments in CLN1 and CLN3 disease; *Ppt1*$^{-/-}$ microglia are morphologically more activated with increased secretion of IL-1β (65), whereas *Cln3*$^{-/-}$ microglia exhibit attenuated morphological responses to pharmacological stimulation with reduced secretion of several chemokines (66). Notably, when *Ppt1*$^{-/-}$ astrocytes and microglia were co-cultured, they appeared to cross-prime one another to exacerbate neuron loss (65), implicating the involvement of astrocyte-microglia crosstalk in CLN1 disease pathophysiology.

Recent research has been delineating the complex and heterogeneous state of activated microglia, a topic that is still under debate. The classification of pro-inflammatory "M1" microglia vs. anti-inflammatory "M2" microglia using the expression of particular cell surface markers and cytokines had been long recognized (57, 84), despite the validity of such a classification still being under scrutiny. M1 polarization of microglia with upregulation of *CD16/32* and *CD86* has been reported in *Ppt1*$^{-/-}$ and *Cln3*$^{-/-}$ mice, and knocking out of the inflammation-related cell adhesion molecule sialoadhesin in those mice attenuated numbers of M1-polarized microglia, levels of pro-inflammatory cytokines, and altered disease phenotype (85). However, given criticism that the M1/M2 dichotomy provides an oversimplified perspective (86, 87), a new pathological classification that incorporates the concept of disease-associated microglia (DAM) has recently been put forth (58, 88). DAM are molecularly characterized by the expression of typical microglial genes such as *Iba1*, *Cst3*, and *Hexb*, coincident with downregulation of homeostatic microglial genes including *P2ry12*, *P2ry13*, *Cx3cr1*, *CD33*, and *Tmem119* (89). DAM further display upregulation of genes involved in lysosomal, phagocytic, and lipid metabolism pathways such as *Apoe*, *Ctsd*, *Lpl*, *Tyrobp*, and *Trem2*, which perhaps makes the DAM classification particularly pertinent to LSDs. RNA sequencing data has revealed the existence of both TREM2-independent and TREM2-dependent DAM in *Tpp1*$^{-/-}$ mice, suggesting the pro-inflammatory and neurotoxic role of activated microglia in CLN2 disease (75, 90). However, the pathological role of DAM still remains debatable; several recent studies have shown neuroprotective effects of TREM2-dependent DAM in mouse models of AD and GRN haploinsufficiency-causing frontotemporal lobar degeneration (GRN-FTLD) (91, 92), suggesting the pathological contribution of DAM may well be disease-dependent. Interestingly, complete deficiency of *Grn*$^{-/-}$ is known to cause CLN11 disease (31), suggesting a similar phenotype may exist in some forms of NCL. Therefore, caution should be exercised in overinterpreting data for the expression of, or staining for, DAM markers and it will be wise not to solely rely on such findings when interpreting pathological roles of activated microglia in NCL pathogenesis in future studies.

The secretion of cytokines and chemokines is of paramount importance for both astrocytes and microglia to exert pro- and anti-inflammatory effects on the process of neurodegeneration (93). The progressive elevation of multiple cytokines and chemokines has been confirmed by whole transcriptomics and/or proteomics in the forebrains and cerebella of *Tpp1*$^{-/-}$ mice (75, 90) and forebrains and spinal cords of *Ppt1*$^{-/-}$ mice (68, 94, 95). Such evidence for the region- and subtype-specific nature of neuroinflammatory changes in CLN1 and CLN2 diseases correlates with the previously shown region- and subtype-specific immunoreactivity of astrogliosis and microglial activation markers. Pharmacological modulation of neuroinflammation is an emerging therapeutic strategy for neurodegenerative diseases (96). Until now, only a few anti-inflammatory drugs have been preclinically tested for NCLs: fingolimod, teriflunomide, and MW151 in *Ppt1*$^{-/-}$ mice (97, 98) and ibuprofen and mycophenolate motefil in *Cln3*$^{-/-}$ deficient mice (99, 100) and provide only partial phenotypic rescue. While modulation of neuroinflammation may provide additional therapeutic benefit, especially when used in combination with other therapies such as ERT or gene therapy, these preclinical results suggest that alteration of central pro-inflammatory cascades in NCL mice might be a non-specific downstream consequence.

Other non-immune-related properties of microglia also have a significant impact on neuronal health. Microglial-mediated

phagocytosis is critical in maintaining CNS homeostasis by pruning synapses or phagocytizing dysfunctional, dying or the debris of deceased neurons and other cell types (57, 101). It has been shown that impaired microglial phagocytic function promotes the development of several neurological diseases such as Rett syndrome (102) and tuberous sclerosis complex (103). Since phagocytosis requires focal exocytosis of lysosomes (104), it is plausible to speculate that lysosomal dysfunction due to NCL protein deficiency could also impair phagocytosis in these cells. While there have been several pieces of evidence from RNA sequencing or proteomics analysis suggesting altered phagocytosis in the brains of *Ppt1*$^{-/-}$ and *Tpp1*$^{-/-}$ mice (75, 94), microglial-specific alteration of phagocytosis is yet to be elucidated. A better understanding of the nature of such dysfunctional phagocytosis by microglia and its contribution to NCL pathogenesis may therefore inform us of new therapeutic targets.

Oligodendrocytes and Schwann Cells

Demyelination is another pathological change widely seen in multiple neurodegenerative diseases. Consistent with recent evidence suggesting the regulatory roles of lysosomal exocytosis in myelination, abnormal myelination is commonly seen in many LSDs including Niemann-Pick disease, Gaucher disease, metachromatic leukodystrophy, multiple sulfatase deficiency, and Krabbe disease (105–107). In contrast, pathological evidence of either dysmyelination or demyelination in the NCLs has been investigated only in mouse models of CLN1, CLN5, and CLN8 diseases with limited depth of characterization (**Table 1**). A key question is whether overt demyelination occurs at all in these disorders, or whether any changes in myelin composition occur secondary to loss of axons, as a result of neuron loss. Certainly, changes in white matter volume are evident in both animal models and human autopsy specimens (6, 21, 29), but its basis is poorly understood. Of course, any consideration of myelin must necessarily include Schwann cells in the peripheral nervous system (PNS), which serve a similar, but not identical role to oligodendrocytes in the CNS. However, the pathological impact of the NCLs upon the PNS is largely underappreciated, but is currently of renewed interest.

CONTRIBUTION OF GLIA TO NCL PATHOGENESIS

A key question that remains to be answered is whether or not the loss of NCL proteins from glial cells confers any direct cell-autonomous effects on these glial cells themselves and/or non-cell-autonomous effects on other cell types including neurons in either a harmful or protective manner. In *in vitro* studies using primary astrocytes, neuron-glial co-culture experiments showed that both *Ppt1*$^{-/-}$ and *Cln3*$^{-/-}$ glia are detrimental to the survival of both wild-type and mutant neurons (65, 66). Such data raise the possibility that mutant astrocytes and microglia may actively trigger the neurodegenerative changes seen in CLN1 and CLN3 diseases. Such *in vitro* models are a crucial component in unraveling cell-type-specific contributions to disease pathogenesis and lend themselves to high throughput screening to detect novel phenotypes and assess potential therapeutic interventions (108–110). Using this approach has highlighted disease-modifying pathways in a number of neurodegenerative diseases that may provide valuable therapeutic targets. Furthermore, the advent of induced pluripotent stem cell (iPSC) models allows the close physiological representation of disease-affected cells on a species-specific genetic background. iPSC models have only been used to a limited extent in the NCLs to date and have so far not been used to generate glial cells despite the availability of well-established differentiation protocols (111–113). For the NCLs, it will be vital to further investigate glial phenotypes *in vitro* and to validate those findings by generating cell-type-specific mutant mice to explore these issues *in vivo*.

Microglial depletion using CSF-1R inhibitors has enabled us to study the direct effect of microglia on the CNS disease process in mammalian models (114). With this technique, it has been shown that microglial depletion in *Ppt1*$^{-/-}$ mice attenuated optic nerve pathologies and several behavioral abnormalities (115). Although such findings might be confounded by the fact that completely abolishing microglia is likely to negatively impact CNS homeostasis, such studies still provide a degree of mechanistic insight into microglial contributions to CLN1 disease progression. Since the effectiveness and safety of some CSF-1R inhibitors have been proven in humans (114) and as new and more specific CSF-1R inhibitors become available, microglial depletion may be a clinically relevant approach.

The *Cre-LoxP* system in mice has proved a powerful tool to investigate the effect of cell-type-specific genetic mutation on neurodegeneration and applied to a wide range of diseases including LSDs *in vivo*. For example, it has been shown that astrocytic-specific deletion of Sulfatase Modifying Factor 1 (SUMF1) (*Sumf1*$^{flox/flox}$; *GFAP-Cre*) was sufficient to induce neuron loss in a mouse model of multiple sulfatase deficiency (MSD) (116). Also, microglial-specific deletion of NPC1 (*Npc1*$^{flox/-}$; *Cx3cr1-Cre*) has been shown to enhance microglial phagocytotic uptake and impaired lipid trafficking, resulting in impaired myelin turnover in a mouse model of Niemann-Pick type C (NPC) disease (117), caused by a deficiency in the NPC1 protein. In contrast, it has also been shown that astrocytic-specific deletion of NPC1 (*Npc1*$^{flox/-}$; *GFAP-CreER*) does not cause neurodegeneration, but neuron-specific knockout (*Npc1*$^{flox/-}$; *Syn1-Cre*) does in the NPC mouse model (118). Such data suggest that the nature of the glial contribution to pathogenesis is likely to differ between LSDs. However, no study has yet investigated the effect of astrocyte-, microglial-, or oligodendrocyte-specific deletion of NCL genes *in vivo* has been reported, indicating that NCL research regarding glial pathology is admittedly lagging behind other LSDs. Perhaps this is in part because of the sheer body of work this would entail given the number of NCL subtypes, as well as the fact that several of the genes that are deficient in the NCLs are lysosomal enzymes that are normally secreted and can be taken up by neighboring cells via a variety of receptor subtypes (42). This process of "cross-correction" naturally confounds and complicates any attempts to generate cell-type-specific PPT1 or TPP-1 deficient mice.

However, recent work in creating chimeric "tethered" versions of enzymes might indeed enable the creation of conditional cell-type-specific models (119).

CONCLUSIONS AND FUTURE DIRECTIONS

Our relatively poor understanding of the pathomechanisms that operate in the NCLs has certainly hampered the generation of more effective therapeutic strategies. Until recently, glial cells across various neurodegenerative diseases have often been considered as poorly defined passive structural elements. The underappreciated consideration of glial involvement in the NCLs is no exception, which is perhaps reflected by the re-naming of these disorders in the 1960s as "neuronal ceroid lipofuscinoses" (120) to distinguish them from other childhood encephalopathies. The rapidly expanding body of research into normal glial biology and their responses to disease has facilitated a reassessment that glia are not just passive bystanders of pathology in the CNS, but instead are active determinants of neurodegeneration. As summarized in this review, there is substantial evidence suggesting such glial involvement in NCL pathophysiology, and changes in glial activation are frequently used to evaluate therapeutic efficacy in preclinical studies (4, 11, 15, 23, 26, 34, 43–47, 97). Of necessity, this review focusses primarily upon the three most common forms of NCL, CLN1 disease, CLN2 disease and CLN3 disease, in which the issue of glial contribution to pathogenesis has been considered. Nevertheless, as detailed in **Table 1**, glial activation is present in all forms of NCL and is consistently present before neuron loss occurs. As such, we might anticipate that glia may also be involved in the pathogenesis of these other forms of NCL. However, given the pronounced difference between even CLN1, CLN2 and CLN3 disease that are discussed in this review, it could be expected that the extent and nature of glial involvement may also vary markedly between types of NCL. Nevertheless, although the glial contribution to disease progression has been intensively studied in other neurodegenerative diseases, relatively little is known about whether glia contribute mechanistically to the profoundly neurodegenerative phenotype of most forms of NCL.

There are several remaining issues that still need addressing in order to clarify the contribution of glial pathology in the NCLs. First, all of the many of subcellular alterations known to be associated with NCLs and other LSDs such as impaired autophagy, lysosomal trafficking, and alterations in the mTOR and TFEB signaling pathways have primarily been studied in neurons or fibroblasts, but not specifically in glial cells of any variety (27, 104, 121–126). Indeed, there is considerable potential that studying these pathways in NCL glia will yield valuable mechanistic information about cell-type-specific impacts of disease-causing mutations. Second, while NCL research has predominantly relied on mouse models, recent evidence has suggested species-dependent differences in the functional properties of astrocytes, questioning the translational relevance of information mouse astrocytes (127). As this issue almost certainly applies to microglia and oligodendrocytes as well, the implementation of glia differentiated from human NCL-patient-derived iPSCs is likely to be of considerable benefit (113). Third, as already discussed, studying the cell-autonomous effects of soluble enzyme deficiency *in vivo* is hampered by "cross-correction," a phenomenon via which mannose 6-phosphate receptor-mediated endocytosis facilitates extracellularly delivered lysosomal enzymes to be taken up by recipient cells. As a previous example of the way to overcome this challenge, the chimeric GALC enzyme tethered to the lysosomal membrane has been engineered in the Krabbe disease mouse model so the cell-autonomous effect of oligodendrocyte-specific GALC deficiency could be studied (119). It will be important to extend such methodology to PPT1 and TPP1 in order to address the cellular autonomy of CLN1 and CLN2 diseases, respectively.

Modern "omics technologies have greatly contributed to a better understanding of the complex physiological nature of glial pathologies in the NCLs and other LSDs (128, 129). RNA sequencing has been widely used in the field of NCLs now that its cost is substantially reduced, but there are a number of caveats concerning the validity of RNA sequencing results. For example, RNA sequencing of a bulk tissue cannot distinguish molecular events in different cell types. As such distinct molecular changes that occur in specific glial cell populations such as microglia and oligodendrocytes, which comprise a relatively small proportion of the total cells present in these samples, might be masked. The application of the single-cell or single-nucleus RNA sequencing technology can theoretically overcome this issue (101), and is likely to reveal new insights into the broad range of effects upon glia in the NCLs. Another issue, which is perhaps unique in LSD research, is that lysosomal proteins play a crucial role in post-translational modification and intracellular trafficking (104, 130), which transcriptomics analyses cannot address. Proteomics analysis instead is more suitable in this case, but again, proteomic data obtained from bulk tissue cannot distinguish between different cell types. Most recently, single-cell proteomics technologies have been invented (131), and it may be predicted that this approach will be widely used to study glial biology in near future.

Notably, glia also exist outside the CNS in different forms depending on the anatomical region. Schwann cells are the myelinating cells in the peripheral nervous system (PNS) and are involved in maintaining ionic balance and providing support to axons (132). There are also non-myelinating Schwann cells called terminal Schwann cells, residing at the neuromuscular junction (133). Satellite glial cells are found in peripheral ganglia and potentially have similar functions to astrocytes in the CNS (134). There is also a unique population of astrocyte-like cells called enteric glial cells, involved in the regulation of the intestinal epithelial barrier and in regulating the function of neurons within the enteric nervous system (ENS) (135). Given the accumulated evidence for glial abnormalities across multiple forms of NCL, it will be important to investigate the impact of disease upon these "non-CNS glial cells" that are key components of the PNS and ENS. These may represent important cellular targets to obtain better therapeutic outcomes in patients with NCLs.

To conclude, much like the different types of musicians in a band that need to coordinate together with its singer

to produce harmonious music, different glial cells provide coordinated support for neuronal health. As in a band it only takes one member to perform sub-optimally for the music to be compromised, and it is very likely that the dysfunction of any one type (or types) of glia similarly contribute to neurodegeneration. With recent technical advances, we are now entering an exciting time for expanding our knowledge of glial dysfunction and its contribution to the pathogenesis of the NCLs. This knowledge will almost certainly help us design more effective and appropriately targeted therapeutic strategies for these disorders.

AUTHOR CONTRIBUTIONS

KT: conceptualization, investigation, and writing the original draft. HN and JL: writing, reviewing, and editing. JC: supervision, conceptualization, reviewing, and editing. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Kingma SDK, Bodamer OA, Wijburg FA. Epidemiology and diagnosis of lysosomal storage disorders; Challenges of screening. *Best Pract Res Clin Endocrinol Metab.* (2015) 29:145–57. doi: 10.1016/j.beem.2014.08.004
2. Mole SE, Cotman SL. Genetics of the neuronal ceroid lipofuscinoses (Batten disease). *Biochim Biophys Acta.* (2015) 1852:2237–41. doi: 10.1016/j.bbadis.2015.05.011
3. Mole SE, Anderson G, Band HA, Berkovic SF, Cooper JD, Kleine Holthaus SM, et al. Clinical challenges and future therapeutic approaches for neuronal ceroid lipofuscinosis. *Lancet Neurol.* (2019) 18:107–16. doi: 10.1016/S1474-4422(18)30368-5
4. Shyng C, Nelvagal HR, Dearborn JT, Tyynelä J, Schmidt RE, Sands MS, et al. Synergistic effects of treating the spinal cord and brain in CLN1 disease. *Proc Natl Acad Sci U S A.* (2017) 114:E5920–9. doi: 10.1073/pnas.1701832114
5. Bible E, Gupta P, Hofmann SL, Cooper JD. Regional and cellular neuropathology in the palmitoyl protein thioesterase-1 null mutant mouse model of infantile neuronal ceroid lipofuscinosis. *Neurobiol Dis.* (2004) 16:346–59. doi: 10.1016/j.nbd.2004.02.010
6. Nelvagal HR, Dearborn JT, Ostergaard JR, Sands MS, Cooper JD. Spinal manifestations of CLN1 disease start during the early postnatal period. *Neuropathol Appl Neurobiol.* (2020) 47:251–67. doi: 10.1111/nan.12658
7. Kielar C, Maddox L, Bible E, Pontikis CC, Macauley SL, Griffey MA, et al. Successive neuron loss in the thalamus and cortex in a mouse model of infantile neuronal ceroid lipofuscinosis. *Neurobiol Dis.* (2007) 25:150–62. doi: 10.1016/j.nbd.2006.09.001
8. Jalanko A, Vesa J, Manninen T, Von Schantz C, Minye H, Fabritius AL, et al. Mice with Ppt1 Δex4 mutation replicate the INCL phenotype and show an inflammation-associated loss of interneurons. *Neurobiol Dis.* (2005) 18:226–41. doi: 10.1016/j.nbd.2004.08.013
9. Bouchelion A, Zhang Z, Li Y, Qian H. Mukherjee AB. Mice homozygous for c451C>T mutation in Cln1 gene recapitulate INCL phenotype. *Ann Clin Transl Neurol.* (2014) 1:1006–23. doi: 10.1002/acn3.144
10. Miller JN, Kovács AD, Pearce DA. The novel Cln1R151X mouse model of infantile neuronal ceroid lipofuscinosis (INCL) for testing nonsense suppression therapy. *Hum Mol Genet.* (2015) 24:185–96. doi: 10.1093/HMG/DDU428
11. Chang M, Cooper JD, Sleat DE, Cheng SH, Dodge JC, Passini MA, et al. Intraventricular enzyme replacement improves disease phenotypes in a mouse model of late infantile neuronal ceroid lipofuscinosis. *Mol Ther.* (2008) 16:649–56. doi: 10.1038/mt.2008.9
12. Ghosh A, Rangasamy SB, Modi KK, Pahan K. Gemfibrozil, food and drug administration-approved lipid-lowering drug, increases longevity in mouse model of late infantile neuronal ceroid lipofuscinosis. *J Neurochem.* (2017) 141:423–35. doi: 10.1111/jnc.13987
13. Geraets RD, Langin LM, Cain JT, Parker CM, Beraldi R, Kovacs AD, et al. tailored mouse model of CLN2 disease: a nonsense mutant for testing personalized therapies. *PLoS ONE.* (2017) 12:e0176526. doi: 10.1371/journal.pone.0176526
14. Pontikis CC, Cella C V, Parihar N, Lim MJ, Chakrabarti S, Mitchison HM, et al. Late onset neurodegeneration in the Cln3 -/- mouse model of juvenile neuronal ceroid lipofuscinosis is preceded by low level glial activation. *Brain Res.* (2004) 1023:231–42. doi: 10.1016/j.brainres.2004.07.030
15. Kovács AD, Saje A, Wong A, Ramji S, Cooper JD, Pearce DA. Age-dependent therapeutic effect of memantine in a mouse model of juvenile Batten disease. *Neuropharmacology.* (2012) 63:769–75. doi: 10.1016/J.NEUROPHARM.2012.05.040
16. Pontikis CC, Cotman SL, MacDonald ME, Cooper JD. Thalamocortical neuron loss and localized astrocytosis in the Cln3Δex7/8 knock-in mouse model of Batten disease. *Neurobiol Dis.* (2005) 20:823–36. doi: 10.1016/j.nbd.2005.05.018
17. Cotman SL. Cln3 Deltaex7/8 knock-in mice with the common JNCL mutation exhibit progressive neurologic disease that begins before birth. *Hum Mol Genet.* (2002) 11:2709–21. doi: 10.1093/hmg/11.22.2709
18. Langin L, Johnson TB, Kovács AD, Pearce DA, Weimer JM, A. tailored Cln3 Q352X mouse model for testing therapeutic interventions in CLN3 Batten disease. *Sci Rep.* (2020) 10:1–12. doi: 10.1038/s41598-020-67478-5
19. Alevy J, Burger CA, Albrecht NE, Jiang D, Samuel MA. Progressive myoclonic epilepsy-associated gene Kctd7 regulates retinal neurovascular patterning and function. *Neurochem Int.* (2019) 129:104486. doi: 10.1016/J.NEUINT.2019.104486

20. von Schantz C, Kielar C, Hansen SN, Pontikis CC, Alexander NA, Kopra O, et al. Progressive thalamocortical neuron loss in Cln5 deficient mice: Distinct effects in Finnish variant late infantile NCL. *Neurobiol Dis.* (2009) 34:308–19. doi: 10.1016/j.nbd.2009.02.001
21. Schmiedt ML, Blom T, Blom T, Kopra O, Wong A, von Schantz-Fant C, et al. Cln5-deficiency in mice leads to microglial activation, defective myelination and changes in lipid metabolism. *Neurobiol Dis.* (2012) 46:19–29. doi: 10.1016/j.nbd.2011.12.009
22. Kopra O, Vesa J, von Schantz C, Manninen T, Minye H, Fabritius AL, et al. A mouse model for Finnish variant late infantile neuronal ceroid lipofuscinosis, CLN5, reveals neuropathology associated with early aging. *Hum Mol Genet.* (2004) 13:2893–906. doi: 10.1093/HMG/DDH312
23. White KA, Nelvagal HR, Poole TA, Lu B, Johnson TB, Davis S, 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. doi: 10.1016/j.omtm.2020.12.014
24. Thelen M, Dαμμe M, Schweizer M, Hagel C, Wong AMS, Cooper JD, et al. Disruption of the autophagy-lysosome pathway is involved in neuropathology of the nclf mouse model of neuronal ceroid lipofuscinosis. *PLoS ONE.* (2012) 7:e35493. doi: 10.1371/journal.pone.0035493
25. Damme M, Brandenstein L, Fehr S, Jankowiak W, Bartsch U, Schweizer M, et al. Gene disruption of Mfsd8 in mice provides the first animal model for CLN7 disease. *Neurobiol Dis.* (2014) 65:12–24. doi: 10.1016/J.NBD.2014.01.003
26. Chen X, Dong T, Hu Y, Shaffo FC, Belur NR, Mazzulli JR, Gray SJ. AAV9/MFSD8 gene therapy is effective in preclinical models of neuronal ceroid lipofuscinosis type 7 disease. *J Clin Invest.* (2022) doi: 10.1172/JCI146286
27. Brandenstein L, Schweizer M, Sedlacik J, Fiehler J, Storch S. Lysosomal dysfunction and impaired autophagy in a novel mouse model deficient for the lysosomal membrane protein Cln7. *Hum Mol Genet.* (2016) 25:777–91. doi: 10.1093/HMG/DDV615
28. Kuronen M, Lehesjoki AE, Jalanko A, Cooper JD, Kopra O. Selective spatiotemporal patterns of glial activation and neuron loss in the sensory thalamocortical pathways of neuronal ceroid lipofuscinosis 8 mice. *Neurobiol Dis.* (2012) 47:444–57. doi: 10.1016/j.nbd.2012.04.018
29. Kuronen M, Hermansson M, Manninen O, Zech I, Talvitie M, Laitinen T, et al. Galactolipid deficiency in the early pathogenesis of neuronal ceroid lipofuscinosis model Cln8mnd: implications to delayed myelination and oligodendrocyte maturation. *Neuropathol Appl Neurobiol.* (2012) 38:471–86. doi: 10.1111/J.1365-2990.2011.01233.X
30. Partanen S, Haapanen A, Kielar C, Pontikis C, Alexander N, Inkinen T, et al. Synaptic changes in the thalamocortical system of cathepsin D-deficient mice. *J Neuropathol Exp Neurol.* (2008) 67:16–29. doi: 10.1097/nen.0b013e31815f3899
31. Ghoshal N, Dearborn JT, Wozniak DF, Cairns NJ. Core features of frontotemporal dementia recapitulated in progranulin knockout mice. *Neurobiol Dis.* (2012) 45:395–408. doi: 10.1016/J.NBD.2011.08.029
32. Kett LR, Stiller B, Bernath MM, Tasset I, Blesa J, Jackson-Lewis V, et al. α-Synuclein-independent histopathological and motor deficits in mice lacking the endolysosomal parkinsonism protein Atp13a2. *J Neurosci.* (2015) 35:5724. doi: 10.1523/JNEUROSCI.0632-14.2015
33. Smith KR, Dahl HHM, Canafoglia L, Andermann E, Damiano J, Morbin M, et al. Cathepsin F mutations cause Type B Kufs disease, an adult-onset neuronal ceroid lipofuscinosis. *Hum Mol Genet.* (2013) 22:1417–23. doi: 10.1093/HMG/DDS558
34. Passini MA, Dodge JC, Bu J, Yang W, Zhao Q, Sondhi D, et al. Intracranial delivery of CLN2 reduces brain pathology in a mouse model of classical late infantile neuronal ceroid lipofuscinosis. *J Neurosci.* (2006) 26:1334–42. doi: 10.1523/JNEUROSCI.2676-05.2006
35. Vuillemenot BR, Katz ML, Coates JR, Kennedy D, Tiger P, Kanazono S, et al. Intrathecal tripeptidyl-peptidase 1 reduces lysosomal storage in a canine model of late infantile neuronal ceroid lipofuscinosis. *Mol Genet Metab.* (2011) 104:325–37. doi: 10.1016/j.ymgme.2011.06.018
36. Vuillemenot BR, Kennedy D, Cooper JD, Wong AMS, Sri S, Doeleman T, et al. Nonclinical evaluation of CNS-administered TPP1 enzyme replacement in canine CLN2 neuronal ceroid lipofuscinosis. *Mol Genet Metab.* (2015) 114:281–93. doi: 10.1016/j.ymgme.2014.09.004
37. Schulz A, Ajayi T, Specchio N, de Los Reyes E, Gissen P, Ballon D, et al. Study of intraventricular cerliponase alfa for CLN2 disease. *N Engl J Med.* (2018) 378:1898–907. doi: 10.1056/NEJMoa1712649
38. Cooper JD, Puhl AC, Wang SH, Eultgen EM, Takahashi K, Le SQ, et al. Devising effective enzyme replacement therapy for infantile onset neuronal ceroid lipofuscinosis (CLN1 disease). *Mol Genet Metab.* (2021) 132:S28. doi: 10.1016/J.YMGME.2020.12.048
39. Markham A. Cerliponase alfa: first global approval. *Drugs.* (2017) 77:1247–9. doi: 10.1007/s40265-017-0771-8
40. Lenders M, Stypmann J, Duning T, Schmitz B, Brand SM, Brand E. Serum-mediated inhibition of enzyme replacement therapy in fabry disease. *J Am Soc Nephrol.* (2016) 27:256–64. doi: 10.1681/ASN.2014121226
41. Rastall DPW, Amalfitano A. Recent advances in gene therapy for lysosomal storage disorders. *Appl Clin Genet.* (2015) 8:157. doi: 10.2147/TACG.S57682
42. Biffi A. Gene therapy for lysosomal storage disorders: a good start. *Hum Mol Genet.* (2016) 25:R65–75. doi: 10.1093/hmg/ddv457
43. Sondhi D, Hackett NR, Peterson DA, Stratton J, Baad M, Travis KM, et al. Enhanced survival of the LINCL mouse following CLN2 gene transfer using the rh10 rhesus Macaque-derived Adeno-associated virus vector. *Mol Ther.* (2007) 15:481–91. doi: 10.1038/sj.mt.6300049
44. Cabrera-Salazar MA, Roskelley EM, Bu J, Hodges BL, Yew N, Dodge JC, et al. Timing of therapeutic intervention determines functional and survival outcomes in a mouse model of late infantile batten disease. *Mol Ther.* (2007) 15:1782–8. doi: 10.1038/sj.mt.6300249
45. Katz ML, Tecedor L, Chen Y, Williamson BG, Lysenko E, Wininger FA, 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) 7:313ra180. doi: 10.1126/scitranslmed.aac6191
46. Kleine Holthaus SM, Aristorena M, Maswood R, Semenyuk O, Hoke J, Hare A, et al. Gene therapy targeting the inner retina rescues the retinal phenotype in a mouse model of CLN3 batten disease. *Hum Gene Ther.* (2020) 31:709. doi: 10.1089/HUM.2020.038
47. Pike LS, Tannous BA, Deliolanis NC, Hsich G, Morse D, Tung CH, et al. Imaging gene delivery in a mouse model of congenital neuronal ceroid lipofuscinosis. *Gene Ther.* (2011) 18:1173–8. doi: 10.1038/gt.2011.118
48. Worgall S, Sondhi D, Hackett NR, Kosofsky B, Kekatpure M V, Neyzi N, et al. Treatment of late infantile neuronal ceroid lipofuscinosis by CNS administration of a serotype 2 adeno-associated virus expressing CLN2 cDNA. *Hum Gene Ther.* (2008) 19:463–74. doi: 10.1089/hum.2008.022
49. Sondhi D, Kaminsky SM, Hackett NR, Pagovich OE, Rosenberg JB, De BP, et al. Slowing late infantile Batten disease by direct brain parenchymal administration of a rh10 adeno-associated virus expressing CLN2. *Sci Transl Med.* (2020) 12:5413. doi: 10.1126/SCITRANSLMED.ABB5413
50. Johnson TB, Cain JT, White KA, Ramirez-Montealegre D, Pearce DA, Weimer JM. Therapeutic landscape for Batten disease: current treatments and future prospects. *Nat Rev Neurol.* (2019) 15:161–78. doi: 10.1038/s41582-019-0138-8
51. Tyynelä J, Cooper JD, Khan MN, Shemilt SJ, Haltia M. Hippocampal pathology in the human neuronal ceroid-lipofuscinoses: distinct patterns of storage deposition, neurodegeneration and glial activation. *Brain Pathol.* (2006) 14:349–57. doi: 10.1111/j.1750-3639.2004.tb00077.x
52. Anderson GW, Goebel HH, Simonati A. Human pathology in NCL. *Biochim Biophys Acta - Mol Basis Dis.* (2013) 1832:1807–26. doi: 10.1016/j.bbadis.2012.11.014
53. Nelvagal HR, Lange J, Takahashi K, Tarczyluk-Wells MA, Cooper JD. Pathomechanisms in the neuronal ceroid lipofuscinoses. *Biochim Biophys Acta - Mol Basis Dis.* (2020) 1866:165570. doi: 10.1016/j.bbadis.2019.165570
54. Morgan JP, Magee H, Wong A, Nelson T, Koch B, Cooper JD, et al. Murine model of variant late infantile ceroid lipofuscinosis recapitulates behavioral and pathological phenotypes of human disease. *PLoS ONE.* (2013) 8:e78694. doi: 10.1371/journal.pone.0078694
55. Bélanger M, Magistretti PJ. The role of astroglia in neuroprotection. *Dialogues Clin Neurosci.* (2009) 11:281–95. doi: 10.31887/DCNS.2009.11.3/mbelanger
56. Chung W-S, Allen NJ, Eroglu C. Astrocytes control synapse formation, function, and elimination. *Cold Spring Harb Perspect Biol.* (2015) 7:a020370. doi: 10.1101/cshperspect.a020370

57. Chen Z, Trapp BD. Microglia and neuroprotection. *J Neurochem.* (2016) 136:10–7. doi: 10.1111/jnc.13062
58. Bennett ML, Viaene AN. What are activated and reactive glia and what is their role in neurodegeneration? *Neurobiol Dis.* (2021) 148:105172. doi: 10.1016/J.NBD.2020.105172
59. Sheeler C, Rosa JG, Ferro A, McAdams B, Borgenheimer E, Cvetanovic M. Glia in neurodegeneration: the housekeeper, the defender and the perpetrator. *Int J Mol Sci.* (2020) 21:1–16. doi: 10.3390/IJMS21239188
60. Sofroniew M V. Astrocyte barriers to neurotoxic inflammation. *Nat Rev Neurosci.* (2015) 16:249–63. doi: 10.1038/nrn3898
61. Prinz M, Jung S, Priller J. Microglia biology: one century of evolving concepts. *Cell.* (2019) 179:292–311. doi: 10.1016/j.cell.2019.08.053
62. Liddelow SA, Barres BA. Reactive Astrocytes: Production, Function, and Therapeutic Potential. *Immunity.* (2017) 46:957–67. doi: 10.1016/j.immuni.2017.06.006
63. Wilkinson FL, Holley RJ, Langford-Smith KJ, Badrinath S, Liao A, Langford-Smith A, et al. Neuropathology in mouse models of mucopolysaccharidosis type I, IIIA and IIIB. *PLoS ONE.* (2012) 7:e35787. doi: 10.1371/JOURNAL.PONE.0035787
64. Takahashi K, Le SQ, Kan S, Jansen MJ, Dickson PI, Cooper JD. Neuropathology of murine Sanfilippo D syndrome. *Mol Genet Metab.* (2021) 134:323–9. doi: 10.1016/J.YMGME.2021.11.010
65. Lange J, Haslett LJ, Lloyd-Evans E, Pocock JM, Sands MS, Williams BP, et al. Compromised astrocyte function and survival negatively impact neurons in infantile neuronal ceroid lipofuscinosis. *Acta Neuropathol Commun.* (2018) 6:74. doi: 10.1186/s40478-018-0575-4
66. Parviainen L, Dihanich S, Anderson GW, Wong AM, Brooks HR, Abeti R, et al. Glial cells are functionally impaired in juvenile neuronal ceroid lipofuscinosis and detrimental to neurons. *Acta Neuropathol Commun.* (2017) 5:74. doi: 10.1186/s40478-017-0476-y
67. Yuan W, Lu L, Rao M, Huang Y, Liu CE, Liu S, et al. GFAP hyperpalmitoylation exacerbates astrogliosis and neurodegenerative pathology in PPT1-deficient mice. *Proc Natl Acad Sci U S A.* (2021) 118:e2022261118. doi: 10.1073/PNAS.2022261118/-/DCSUPPLEMENTAL
68. Macauley SL, Pekny M, Sands MS. The role of attenuated astrocyte activation in infantile neuronal ceroid lipofuscinosis. (2011). doi: 10.1523/JNEUROSCI.3579-11.2011
69. Moulson AJ, Squair JW, Franklin RJM, Tetzlaff W, Assinck P. Diversity of reactive astrogliosis in CNS pathology: heterogeneity or plasticity? *Front Cell Neurosci.* (2021) 15:703810. doi: 10.3389/fncel.2021.703810
70. Wu T, Dejanovic B, Gandham VD, Gogineni A, Edmonds R, Schauer S, et al. Complement C3 is activated in human AD brain and is required for neurodegeneration in mouse models of amyloidosis and tauopathy. *Cell Rep.* (2019) 28:2111–23.e6. doi: 10.1016/j.celrep.2019.07.060
71. Guttenplan KA, Weigel MK, Adler DI, Couthouis J, Liddelow SA, Gitler AD, et al. Knockout of reactive astrocyte activating factors slows disease progression in an ALS mouse model. *Nat Commun.* (2020) 11:1–9. doi: 10.1038/s41467-020-17514-9
72. Yun SP, Kam TI, Panicker N, Kim S, Oh Y, Park JS, et al. Block of A1 astrocyte conversion by microglia is neuroprotective in models of Parkinson's disease. *Nat Med.* (2018) 24:931–8. doi: 10.1038/s41591-018-0051-5
73. Sadhukhan T, Bagh MB, Appu AP, Mondal A, Zhang W, Liu A, et al. In a mouse model of INCL reduced S-palmitoylation of cytosolic thioesterase APT1 contributes to microglia proliferation and neuroinflammation. *J Inherit Metab Dis.* (2021) 44:1051–69. doi: 10.1002/jimd.12379
74. Escartin C, Galea E, Lakatos A, O'Callaghan JP, Petzold GC, Serrano-Pozo A, et al. Reactive astrocyte nomenclature, definitions, and future directions. *Nat Neurosci.* (2021) 24:312. doi: 10.1038/S41593-020-00783-4
75. Domowicz MS, Chan W-C, Claudio-Vázquez P, Gonzalez T, Schwartz NB. Brain transcriptome analysis of a CLN2 mouse model as a function of disease progression. *J Neuroinflammation.* (2021) 18:1–18. doi: 10.1186/S12974-021-02302-Z
76. Diaz-Castro B, Gangwani MR, Yu X, Coppola G, Khakh BS. Astrocyte molecular signatures in Huntington's disease. *Sci Transl Med.* (2019) 11:8546. doi: 10.1126/scitranslmed.aaw8546
77. Khakh BS, Sofroniew M V. Diversity of astrocyte functions and phenotypes in neural circuits. *Nat Neurosci.* (2015) 18:942–52. doi: 10.1038/nn.4043
78. Han RT, Kim RD, Molofsky AV, Liddelow SA. Astrocyte-immune cell interactions in physiology and pathology. *Immunity.* (2021) 54:211–24. doi: 10.1016/j.immuni.2021.01.013
79. Galloway DA, Phillips AEM, Owen DRJ, Moore CS. Phagocytosis in the brain: Homeostasis and disease. *Front Immunol.* (2019) 10:790. doi: 10.3389/FIMMU.2019.00790/BIBTEX
80. Jha MK, Jo M, Kim J-H, Suk K. Microglia-astrocyte crosstalk: an intimate molecular conversation. *Neurosci.* (2019) 25:227–40. doi: 10.1177/1073858418783959
81. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature.* (2017) 541:481–7. doi: 10.1038/nature21029
82. Joshi AU, Minhas PS, Liddelow SA, Haileselassie B, Andreasson KI, Dorn GW, et al. Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration. *Nat Neurosci.* (2019) 22:1635–48. doi: 10.1038/s41593-019-0486-0
83. Aguzzi A, Barres BA, Bennett ML. Microglia: scapegoat, saboteur, or something else? *Science.* (2013) 339:156–61. doi: 10.1126/SCIENCE.1227901
84. Butovsky O, Weiner HL. Microglial signatures and their role in health and disease. *Nat Rev Neurosci.* (2018) 19:622–35. doi: 10.1038/s41583-018-0057-5
85. Groh J, Ribechini E, Stadler D, Schilling T, Lutz MB, Martini R. Sialoadhesin promotes neuroinflammation-related disease progression in two mouse models of CLN disease. *Glia.* (2016) 64:792–809. doi: 10.1002/glia.22962
86. Ransohoff RM. A polarizing question: do M1 and M2 microglia exist? *Nat Neurosci.* (2016) 19:987–991. doi: 10.1038/nn.4338
87. Jurga AM, Paleczna M, Kuter KZ. Overview of general and discriminating markers of differential microglia phenotypes. *Front Cell Neurosci.* (2020) 0:198. doi: 10.3389/FNCEL.2020.00198
88. Deczkowska A, Keren-Shaul H, Weiner A, Colonna M, Schwartz M, Amit I. Disease-associated microglia: a universal immune sensor of neurodegeneration. *Cell.* (2018) 173:1073–81. doi: 10.1016/J.CELL.2018.05.003
89. Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, et al. Identification of a unique TGF-β-dependent molecular and functional signature in microglia. *Nat Neurosci.* (2013) 17:131–143. doi: 10.1038/nn.3599
90. Domowicz MS, Chan WC, Claudio-Vázquez P, Henry JG, Ware CB, Andrade J, et al. Global brain transcriptome analysis of a Tpp1 neuronal ceroid lipofuscinoses mouse model. *ASN Neuro.* (2019) 11:175909141984339. doi: 10.1177/1759091419843393
91. Lee SH, Meilandt WJ, Xie L, Gandham VD, Ngu H, Barck KH, et al. Trem2 restrains the enhancement of tau accumulation and neurodegeneration by β-amyloid pathology. *Neuron.* (2021) 109:1283–301.e6. doi: 10.1016/J.NEURON.2021.02.010
92. Reifschneider A, Robinson S, Van Lengerich B, Gnörich J, Logan T, Heindl S, et al. Loss of TREM2 rescues hyperactivation of microglia, but not lysosomal deficits and neurotoxicity in models of progranulin deficiency. *EMBO J.* (2022) 14:e109108. doi: 10.15252/EMBJ.2021109108
93. Salvador AF, de Lima KA, Kipnis J. Neuromodulation by the immune system: a focus on cytokines. *Nat Rev Immunol.* (2021) 21:526–41. doi: 10.1038/s41577-021-00508-z
94. Nelvagal HR, Hurtado ML, Eaton SL, Kline RA, Lamont DJ, Sands MS, et al. Comparative proteomic profiling reveals mechanisms for early spinal cord vulnerability in CLN1 disease. *Sci Rep.* (2020) 10:1–16. doi: 10.1038/s41598-020-72075-7
95. Qiao X, Lu J-Y, Hofmann SL. Gene expression profiling in a mouse model of infantile neuronal ceroid lipofuscinosis reveals upregulation of immediate early genes and mediators of the inflammatory response. *BMC Neurosci.* (2007) 8:95. doi: 10.1186/1471-2202-8-95
96. Guzman-Martinez L, Maccioni RB, Andrade V, Navarrete LP, Pastor MG, Ramos-Escobar N. Neuroinflammation as a common feature of neurodegenerative disorders. *Front Pharmacol.* (2019) 10:1008. doi: 10.3389/fphar.2019.01008
97. Groh J, Berve K, Martini R. Immune modulation attenuates infantile neuronal ceroid lipofuscinosis in mice before and after disease onset. *Brain Commun.* (2021) 3: fcab047. doi: 10.1093/BRAINCOMMS/FCAB047
98. Macauley SL, Wong AMS, Shyng C, Augner DP, Dearborn JT, Pearse Y, et al. An anti-neuroinflammatory that targets dysregulated

glia enhances the efficacy of CNS-directed gene therapy in murine infantile neuronal ceroid lipofuscinosis. *J Neurosci.* (2014) 34:13077–82. doi: 10.1523/JNEUROSCI.2518-14.2014
99. Tarczyluk-Wells MA, Salzlechner C, Najafi AR, Lim MJ, Smith D, Platt FM, et al. Combined anti-inflammatory and neuroprotective treatments have the potential to impact disease phenotypes in Cln3−/− mice. *Front Neurol.* (2019) 10:963. doi: 10.3389/FNEUR.2019.00963/BIBTEX
100. Seehafer SS, Ramirez-Montealegre D, Wong AMS, Chan CH, Castaneda J, Horak M, et al. Immunosuppression alters disease severity in juvenile Batten disease mice. *J Neuroimmunol.* (2011) 230:169. doi: 10.1016/J.JNEUROIM.2010.08.024
101. Li Q, Barres BA. Microglia and macrophages in brain homeostasis and disease. *Nat Rev Immunol.* (2018) 18:225–42. doi: 10.1038/nri.2017.125
102. Derecki NC, Cronk JC, Lu Z, Xu E, Abbott SBG, Guyenet PG, et al. Wild-type microglia arrest pathology in a mouse model of Rett syndrome. *Nature.* (2012) 484:105–9. doi: 10.1038/nature10907
103. Zhao X, Liao Y, Morgan S, Mathur R, Feustel P, Mazurkiewicz J, et al. Noninflammatory changes of microglia are sufficient to cause epilepsy. *Cell Rep.* (2018) 22:2080–93. doi: 10.1016/j.celrep.2018.02.004
104. Lie PPY, Nixon RA. Lysosome trafficking and signaling in health and neurodegenerative diseases. *Neurobiol Dis.* (2019) 122:94–105. doi: 10.1016/j.nbd.2018.05.015
105. Faust PL, Kaye EM, Powers JM. Myelin lesions associated with lysosomal and peroxisomal disorders. *Expert Rev Neurother.* (2014) 10:1449–1466. doi: 10.1586/ERN.10.127
106. Chen G. The roles of lysosomal exocytosis in regulated myelination. *J Neurol Neuromedicine.* (2016) 1:4–8. doi: 10.29245/2572.942X/2016/5.1047
107. Graziano ACE, Cardile V. History, genetic, and recent advances on Krabbe disease. *Gene.* (2015) 555:2–13. doi: 10.1016/J.GENE.2014.09.046
108. Little D, Ketteler R, Gissen P, Devine MJ. Using stem cell–derived neurons in drug screening for neurological diseases. *Neurobiol Aging.* (2019) 78:130–41. doi: 10.1016/j.neurobiolaging.2019.02.008
109. Lange J, Wood-Kaczmar A, Ali A, Farag S, Ghosh R, Parker J, et al. Mislocalization of nucleocytoplasmic transport proteins in human Huntington's disease PSC-derived striatal neurons. *Front Cell Neurosci.* (2021) 15:393. doi: 10.3389/FNCEL.2021.742763/BIBTEX
110. Sherman SP, Bang AG. High-throughput screen for compounds that modulate neurite growth of human induced pluripotent stem cell-derived neurons. *Dis Model Mech.* (2018) 11:dmm031906 doi: 10.1242/DMM.031906
111. Sima N, Li R, Huang W, Xu M, Beers J, Zou J, et al. Neural stem cells for disease modeling and evaluation of therapeutics for infantile (CLN1/PPT1) and late infantile (CLN2/TPP1) neuronal ceroid lipofuscinoses. *Orphanet J Rare Dis.* (2018) 13:1–14. doi: 10.1186/S13023-018-0798-2/FIGURES/8
112. Uusi-Rauva K, Blom T, Von Schantz-Fant C, Blom T, Jalanko A, Kyttälä A. Induced pluripotent stem cells derived from a CLN5 patient manifest phenotypic characteristics of neuronal ceroid lipofuscinoses. *Int J Mol Sci.* (2017) 18:955. doi: 10.3390/IJMS18050955
113. Lojewski X, Staropoli JF. Biswas-legrand S, Simas AM, Haliw L, Selig MK, et al. Human iPSC models of neuronal ceroid lipofuscinosis capture distinct effects of TPP1 and CLN3 mutations on the endocytic pathway. *Hum Mol Genet.* (2014) 23:2005–22. doi: 10.1093/HMG/DDT596
114. Green KN, Crapser JD, Hohsfield LA. To kill microglia: a case for CSF1R inhibitors. *Trends Immunol.* (2020) 41:771. doi: 10.1016/J.IT.2020.07.001
115. Berve K, West BL, Martini R, Groh J. Sex- and region-biased depletion of microglia/macrophages attenuates CLN1 disease in mice. *J Neuroinflammation.* (2020) 17:323. doi: 10.1186/s12974-020-01996-x
116. Di Malta C, Fryer JD, Settembre C, Ballabio A. Astrocyte dysfunction triggers neurodegeneration in a lysosomal storage disorder. *Proc Natl Acad Sci U S A.* (2012) 109:E2334–42. doi: 10.1073/pnas.1209577109
117. Colombo A, Dinkel L, Müller SA, Sebastian Monasor L, Schifferer M, Cantuti-Castelvetri L, et al. Loss of NPC1 enhances phagocytic uptake and impairs lipid trafficking in microglia. *Nat Commun.* (2021) 12:1–20. doi: 10.1038/s41467-021-21428-5
118. Yu T, Shakkottai VG, Chung C, Lieberman AP. Temporal and cell-specific deletion establishes that neuronal Npc1 deficiency is sufficient to mediate neurodegeneration. *Hum Mol Genet.* (2011) 20:4440–51. doi: 10.1093/hmg/ddr372
119. Mikulka CR, Dearborn JT, Benitez BA, Strickland A, Liu L, Milbrandt J, et al. Cell-autonomous expression of the acid hydrolase galactocerebrosidase. *Proc Natl Acad Sci.* (2020)201917675. doi: 10.1073/pnas.1917675117
120. Haltia M, Goebel HH. The neuronal ceroid-lipofuscinoses: a historical introduction. *Biochim Biophys Acta - Mol Basis Dis.* (2013) 1832:1795–800. doi: 10.1016/j.bbadis.2012.08.012
121. Seranova E, Connolly KJ, Zatyka M, Rosenstock TR, Barrett T, Tuxworth RI, et al. Dysregulation of autophagy as a common mechanism in lysosomal storage diseases. *Essays Biochem.* (2017) 61:733–49. doi: 10.1042/EBC20170055
122. Chandrachud U, Walker MW, Simas AM, Heetveld S, Petcherski A, Klein M, et al. Identification of autophagy modifiers in Batten disease Unbiased cell-based screening in a neuronal cell model of Batten disease highlights an interaction between Ca 2+ homeostasis, autophagy, and CLN3 function. *J Biol Chem.* (2015) 290:14361–80. doi: 10.1074/jbc.M114.621706
123. Palmieri M, Pal R, Nelvagal HR, Lotfi P, Stinnett GR, Seymour ML, et al. mTORC1-independent TFEB activation via Akt inhibition promotes cellular clearance in neurodegenerative storage diseases. *Nat Commun.* (2017) 8:14338. doi: 10.1038/ncomms14338
124. Bajaj L, Sharma J, di Ronza A, Zhang P, Eblimit A, Pal R, et al. A CLN6-CLN8 complex recruits lysosomal enzymes at the ER for Golgi transfer. *J Clin Invest.* (2020) 140:4118–32. doi: 10.1172/JCI130955
125. Shlevkov E, Basu H, Bray M-A, Sun Z, Wei W, Apaydin K, et al. A high-content screen identifies TPP1 and Aurora B as regulators of axonal mitochondrial transport. *Cell Rep.* (2019) 28:3224. doi: 10.1016/J.CELREP.2019.08.035
126. Danyukova T, Ariunbat K, Thelen M, Brocke-Ahmadinejad N, Mole SE, Storch S. Loss of CLN7 results in depletion of soluble lysosomal proteins and impaired mTOR reactivation. *Hum Mol Genet.* (2018) 27:1711–22. doi: 10.1093/HMG/DDY076
127. Li J, Pan L, Pembroke WG, Rexach JE, Godoy MI, Condro MC, et al. Conservation and divergence of vulnerability and responses to stressors between human and mouse astrocytes. *Nat Commun.* (2021) 12:1–20. doi: 10.1038/s41467-021-24232-3
128. Parenti G, Medina DL, Ballabio A. The rapidly evolving view of lysosomal storage diseases. *EMBO Mol Med.* (2021) 13:e12836. doi: 10.15252/EMMM.202012836
129. Rintz E, Gaffke L, Podlacha M, Brokowska J, Cyske Z, Wegrzyn G, et al. Transcriptomic changes related to cellular processes with particular emphasis on cell activation in lysosomal storage diseases from the group of mucopolysaccharidoses. *Int J Mol Sci.* (2020) 21:3194. doi: 10.3390/IJMS21093194
130. Mukherjee AB, Appu AP, Sadhukhan T, Casey S, Mondal A, Zhang Z, et al. Emerging new roles of the lysosome and neuronal ceroid lipofuscinoses. *Mol Neurodegener.* (2019) 14:4. doi: 10.1186/s13024-018-0300-6
131. Petelski AA, Emmott E, Leduc A, Huffman RG, Specht H, Perlman DH, et al. Multiplexed single-cell proteomics using SCoPE2. *Nat Protoc.* (2021) 16:5398–425. doi: 10.1038/s41596-021-00616-z
132. Kidd GJ, Ohno N, Trapp BD. Biology of schwann cells. *Handb Clin Neurol.* (2013) 115:55–79. doi: 10.1016/B978-0-444-52902-2.00005-9
133. Santosa KB, Keane AM, Jablonka-Shariff A, Vannucci B, Snyder-Warwick AK. Clinical relevance of terminal schwann cells: an overlooked component of the neuromuscular junction. *J Neurosci Res.* (2018) 96:1125. doi: 10.1002/JNR.24231
134. Hanani M, Spray DC. Emerging importance of satellite glia in nervous system function and dysfunction. *Nat Rev Neurosci.* (2020) 21:485. doi: 10.1038/S41583-020-0333-Z
135. Yu YB Li YQ. Enteric glial cells and their role in the intestinal epithelial barrier. *World J Gastroenterol.* (2014) 20:11273. doi: 10.3748/WJG.V20.I32.11273

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Experimental Therapeutic Approaches for the Treatment of Retinal Pathology in Neuronal Ceroid Lipofuscinoses

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The neuronal ceroid lipofuscinoses (NCLs) are a group of childhood-onset neurodegenerative lysosomal storage disorders mainly affecting the brain and the retina. In the NCLs, disease-causing mutations in 13 different ceroid lipofuscinoses genes (CLN) have been identified. The clinical symptoms include seizures, progressive neurological decline, deterioration of motor and language skills, and dementia resulting in premature death. In addition, the deterioration and loss of vision caused by progressive retinal degeneration is another major hallmark of NCLs. To date, there is no curative therapy for the treatment of retinal degeneration and vision loss in patients with NCL. In this review, the key findings of different experimental approaches in NCL animal models aimed at attenuating progressive retinal degeneration and the decline in retinal function are discussed. Different approaches, including experimental enzyme replacement therapy, gene therapy, cell-based therapy, and immunomodulation therapy were evaluated and showed encouraging therapeutic benefits. Recent experimental ocular gene therapies in NCL animal models with soluble lysosomal enzyme deficiencies and transmembrane protein deficiencies have shown the strong potential of gene-based approaches to treat retinal dystrophies in NCLs. In CLN3 and CLN6 mouse models, an adeno-associated virus (AAV) vector-mediated delivery of *CLN3* and *CLN6* to bipolar cells has been shown to attenuate the retinal dysfunction. Therapeutic benefits of ocular enzyme replacement therapies were evaluated in CLN2 and CLN10 animal models. Since brain-targeted gene or enzyme replacement therapies will most likely not attenuate retinal neurodegeneration, there is an unmet need for treatment options additionally targeting the retina in patients with NCL. The long-term benefits of these therapeutic interventions aimed at attenuating retinal degeneration and vision loss in patients with NCL remain to be investigated in future clinical studies.

Keywords: neuronal ceroid lipofuscinoses, NCL, Batten disease, retinal degeneration, enzyme replacement therapy, gene therapy, lysosomal storage disorder

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INTRODUCTION

This review focuses on experimental approaches aimed at attenuating the progression of retinal degeneration in the different animal models of neuronal ceroid lipofuscinoses (NCLs). The NCLs are caused by defects in 13 different genes (CLN1–CLN8, CLN10–CLN14) encoding soluble lysosomal proteins (CLN1, CLN2, CLN5, CLN10, CLN11, and CLN13), membrane proteins located in the ER (CLN6 and CLN8), ER-Golgi intermediate compartment (CLN8) or lysosomes (CLN3, CLN7, and CLN12), and cytosolic proteins associated with synaptic vesicles (CLN4) or the plasma membrane (CLN14, **Table 1**) (1). The majority of affected gene products play important roles for lysosomal biogenesis and function: soluble lysosomal enzymes involved in lysosomal protein degradation (palmitoyl-protein thioesterase 1/PPT1, tripeptidyl-peptidase 1/TPP1, cathepsin D, and cathepsin F), soluble lysosomal proteins with unknown function (CLN5), and polytopic lysosomal membrane proteins (CLN3, CLN7, and CLN12). The primary function of the lysosomal membrane protein CLN3 is unclear (2). CLN7 was recently shown to function as an endolysosomal chloride channel (3). CLN6 and CLN8 form a complex in the endoplasmic reticulum (ER) which is required for the biosynthetic transport of a subset of newly synthesized soluble lysosomal proteins from the ER to the Golgi apparatus (4, 5). Biochemically, defects in NCL genes lead to lysosomal and autophagic dysfunction and subsequent accumulation of autofluorescent ceroid lipopigments. Based on the age of patients at the onset of first symptoms, the NCLs have been classified into congenital, infantile, late infantile, juvenile, and adult NCL phenotypes (6, 7). The clinical symptoms include seizures, progressive neurological decline, deterioration of motor and language skills, and dementia resulting in premature death (8). All disease-causing mutations and sequence variations in the *CLN* genes are summarized in the NCL mutation database (https://www.ucl.ac.uk/ncl-disease), and genotype-phenotype correlations are discussed in a recent review (9). With the exception of CLN4, CLN12, and CLN13 disease, deterioration and loss of vision is another major hallmark of NCLs (**Table 1**) (10). However, the deterioration of vision does not always appear as the first symptom in different NCLs (11). In rare cases, patients with CLN3 and CLN7 disease present with non-syndromic retina degeneration (12–14). To date, there are no curative therapies for the treatment of neurodegeneration in the brain and the retina, and patients rely on palliative treatment (15). An enzyme replacement therapy (ERT) using the intracerebroventricular infusions of recombinant TPP1 (cerliponase alfa) every 2 weeks has been shown to decelerate the disease progression and was recently approved for the treatment of patients with CLN2 disease (16).

Naturally occurring and gene-targeted mouse models and large animal models, such as dogs, sheep, and macaques, allowed the age-dependent morphological, biochemical, and functional analyses of retinal pathologies in NCL (17, 18). The pathomechanisms leading to the degeneration of neuronal cells in different retinal cell layers and the loss of retinal function are not well understood. CLN3 has been suggested to be required for the phagocytosis of photoreceptor outer segments by retinal pigment epithelial (RPE) cells (19). In line with this notion, lysosomal storage and increased numbers of mature autophagosomes and basal phagolysosomes were found in the retinal pigment epithelium of $Cln3^{\Delta ex1-6}$ mice, a CLN3 mouse model (20).

Preclinical studies targeting the brain of NCL animal models using ERT and gene therapy demonstrated a delayed onset and an attenuated progression of neuroinflammation and neurodegeneration (21). However, intracerebroventricular ERT and brain-targeted gene therapies are unlikely to prevent or attenuate neurodegeneration in the retina (15, 22). Therefore a combination of the brain- and eye-directed therapy might be required to prevent neurodegeneration in both the brain and the retina (23).

GENE THERAPY

Gene therapies for lysosomal storage disorders and NCLs are designed to correct the primary genetic defect (21). Experimental adeno-associated virus (AAV) vector-based brain-targeted gene therapies have been evaluated in animal models for CLN1, CLN2, CLN3, CLN5, CLN6, CLN7, CLN8, CLN10, and CLN11 disease (21, 24, 25). Based on the promising results of the preclinical studies, some of these brain-targeted gene therapies are currently being tested in clinical trials in CLN2, CLN3, and CLN6 patients (21). Ocular gene therapy is an emerging field. In general, gene therapies for soluble lysosomal enzyme deficiencies involve the cross-correction of non-transduced cells, and therapeutic benefits might thus be achieved with a relatively low number of successfully transduced cells. Gene therapies for membrane protein deficiencies, in comparison, will most likely require higher numbers of transduced cells to achieve therapeutic benefits (23). The most widely used viral vectors for ocular gene therapy are based on AAVs since they mediate stable, long-term transgene expression, and produce only minor immune responses (26). Preclinical ocular gene therapy studies in NCL animal models have mainly used intravitreally administered AAV vectors with different serotypes prior to the onset of the retinal pathology (27). Ocular gene therapy studies have been performed in animal models targeting soluble lysosomal protein deficiencies (CLN1, CLN5, CLN10, and CLN11) but also transmembrane protein defects (CLN3 and CLN6) (**Table 2**) and showed encouraging results. To date, the long-term therapeutic benefits of ocular gene therapy in human patients with NCL are unknown since no clinical trials are ongoing or have been completed (21).

CLN1 DISEASE

The CLN1 mouse model is characterized by the progressive loss of photoreceptors starting at 3 months of age and decreased retinal functions compared with wild type mice as measured by electroretinogram (ERG) recordings (28). Intravitreal administration of an AAV2 vector carrying human *PPT1* cDNA led to a five-fold increase in PPT1 enzymatic

TABLE 1 | Summary of NCL forms, localization and function of gene products and retinal pathology in human patients.

NCL form	Protein	Localization and function	Clinical phenotypes	Retinal pathology
CLN1	Palmitoyl-protein thioesterase 1 (PPT1)	Lysosomal enzyme Long-chain fatty acyl hydrolase	**Infantile*** Late infantile Juvenile Adult	Loss of vision, progressive retina atrophy, optic nerve atrophy
CLN2	Tripeptidyl-peptidase 1 (TPP1)	Lysosomal enzyme Serine protease	**Late infantile*** Juvenile	Loss of vision, optic nerve atrophy
CLN3	CLN3	Lysosomal membrane protein Unknown	**Juvenile*** Retinitis pigmentosa Adult Cone-rod dystrophy	Loss of vision is leading symptom, macular degeneration, optic nerve atrophy
CLN4	Cysteine string protein α	Cytoplasmic protein-associated with synaptic vesicles Regulation of neurotransmitter release, Exocytosis/endocytosis coupling	**Adult*** (Autosomal-dominant, Parry disease)	Not known
CLN5	CLN5	Lysosomal protein Unknown	**Late infantile*** Juvenile Adult	Retinal degeneration, loss of vision
CLN6	CLN6	ER membrane protein Biosynthetic transport from ER to Golgi	**Late infantile *** Adult	Loss of vision
CLN7	CLN7	Lysosomal membrane protein Chloride channel	**Late infantile*** Juvenile Adult Macular dystrophy Cone-rod dystrophy	Loss of vision as later symptom
CLN8	CLN8	ER/ER-Golgi intermediate compartment membrane protein Biosynthetic transport from ER to Golgi	**Late infantile*** Juvenile	Retinopathy and loss of vision, optic nerve atrophy
CLN10	Cathepsin D (CTSD)	Lysosomal enzyme Aspartic endoprotease	**Congenital*** Late infantile Juvenile Adult	Congenital: unclear Retinopathy and loss of vision for later onset forms
CLN11	Progranulin (PGRN)	Lysosomal enzyme chaperone Neuronal survival and axonal growth factor	**Adult*** Frontotemporal lobar dementia (heterozygous mutation)	Retinopathy and loss of vision
CLN12	ATP13A2	Lysosomal membrane protein Polyamine-transporting ATPase	**Juvenile***	Not known
CLN13	Cathepsin F (CTSF)	Lysosomal enzyme Cysteine protease	**Adult*** (Kufs disease)	Not known
CLN14	BTB/POZ domain-containing protein KCTD7 (KCTD7)	Cytoplasmic protein-partially associated with plasma membrane Unknown	Infantile Late infantile	Loss of vision, optic nerve atrophy

**Phenotypes present in patients with a complete loss of protein function.*

TABLE 2 | Preclinical ocular-targeted gene therapies for NCLs.

NCL type	Animal model	Viral vector	Delivery route	Age of intervention	References
CLN1	*Ppt1* knockout mouse	AAV2	Intravitreal	P18–21 or 8 weeks	(28)
CLN3	*Cln3*$^{\Delta ex7/8}$ knock-in mouse	AAV7m8	Intravitreal	P5 or P6	(29)
		AAV2/8	Subretinal	P14	(29)
CLN5	*CLN5*-deficient Borderdale sheep	AAV9	Intravitreal	3 months	(30)
CLN6	*Cln6/nclf* mouse	AAV7m8	Intravitreal	P5 or P6	(31)
	CLN6-deficient South Hampshire sheep	AAV9	Intravitreal	3 months	(30)
CLN10	*CtsD* knockout mouse	AAVshH10	Intravitreal	P5	(32)
CLN11	*Pgrn* knockout mouse	AAV2.7m8	Intravitreal	1, 6, and 12 months	(33)
		AAV9.2YF	Systemic	P3 or P4	

activities compared with age-matched wild type mice (28). Although a better organization of the photoreceptor layer and improved retinal function as measured by ERG recordings were detected in AAV2-*PPT1* treated mutant mice, the progression of the retinal dystrophy was only retarded but not completely prevented.

CLN2 DISEASE

A naturally occurring *TPP1*-deficient Dachshund model recapitulates the key features of human CLN2 disease, including ataxia, tremor, progressive brain atrophy, loss of vision, and a reduced life span (34). For CLN2 disease, there are no reports on the efficacy of experimental ocular gene therapies in animal models. A single pre-symptomatic intraventricular injection of an AAV2 vector harboring canine *TPP1 (cTPP1)* cDNA into the CLN2 Dachshund model led to the reduced storage of autofluorescent material and decreased astrocytosis in the brain, and delayed onset of cognitive deficits and extended lifespan of the mutant dogs (35). However, TPP1 was not detected in photoreceptors and retinal pigment epithelial cells. In the treated CLN2 mutant dogs, the retinal degeneration and reduction of ERG b-wave amplitudes were not prevented by the brain-directed administration of AAV2-c*TPP1* compared with untreated dogs (36). These data suggest that AAV-mediated brain-targeted gene therapy is not sufficient to treat the retinal degeneration and loss of vision in CLN2 dog models.

CLN3 DISEASE

The *Cln3* knock-in mouse model ($Cln3^{\Delta ex7/8}$) genetically recapitulates the 1 Kb deletion mutation of exons 7 and 8 found in 85% of human patients with CLN3 disease (37). Retinal degeneration in $Cln3^{\Delta ex7/8}$ mice is relatively mild leading to the loss of bipolar cells and a progressive reduction of the b-wave amplitudes beginning from 12 months of age (29, 38). These data demonstrate a progressive reduction of inner retinal function in the retinas of mutant mice. An ocular gene therapy using the intravitreal injections of an AAV7m8 vector harboring human *CLN3* in postnatal $Cln3^{\Delta ex7/8}$ mice led to the improved survival of bipolar cells and retinal function (29). In contrast, a subretinal injection using an AAV2/8-*mCln3* vector targeting photoreceptors and retinal pigment epithelial cells did not attenuate the loss of bipolar cells and the decline in inner retinal function suggesting that the expression of CLN3 in photoreceptors was not therapeutic in the mutant mice. Of note, the $Cln3^{\Delta ex7/8}$ mice do not fully recapitulate the retinal phenotype observed in patients with CLN3 disease. The number of photoreceptors was unchanged in the 15-month-old $Cln3^{\Delta ex7/8}$ mice whereas photoreceptors in the retinal postmortem tissues of patients with CLN3 disease are almost completely lost (7).

CLN5 DISEASE

Murray and colleagues reported on the first successful intravitreal gene therapy in a large NCL animal model (30). They used the naturally occurring *CLN5*-deficient Borderdale sheep which recapitulates the key features of human CLN5 disease, such as motor and cognitive decline, progressive neurodegeneration in the brain and the retina, and loss of vision (39). The sheep eye represents a good model for the human eye because of its similar morphology and size. *CLN5*-deficient Borderdale sheep received a single intravitreal injection of an AAV9 vector harboring ovine *CLN5* cDNA at 3 months of age and were analyzed at 18 months of age (30). AAV9-*CLN5*-treated eyes showed minor lysosomal storage and neuroinflammation and intact retinal layers with a thickness comparable to that of the control sheep (30). In addition, the measurements of retinal functions in treated eyes showed ERG amplitudes nearly comparable with amplitudes in wild type control sheep (30).

CLN6 DISEASE

The *Cln6* ^{nclf} mouse is a naturally occurring mouse model of CLN6 disease (40). The main pathological features in the retina of *Cln6* ^{nclf} mice include the loss of photoreceptors, early-onset reactive gliosis, accumulation of lysosomal storage material in multiple retinal cell layers, and the increased expression of soluble lysosomal enzymes (41). Surprisingly, an AAV2/8 vector-mediated gene transfer of human or mouse *CLN6* did not prevent the loss of photoreceptors and did not preserve photoreceptor functions in *Cln6* ^{nclf} *mice*. In contrast, an AAV2.7m8 vector-mediated bipolar cell-specific expression of *CLN6* prevented the loss of photoreceptors and preserved their function (31). Data indicated that *Cln6* deficiency in bipolar cells is the cause of photoreceptor degeneration in the *Cln6* ^{nclf} mouse. White and colleagues reported for the first time that brain-targeted gene therapy in *Cln6* ^{nclf} mice attenuated retinal pathology. Intracerebroventricular gene therapy in *Cln6* ^{nclf} mice reduced the pathology in visual centers of the brain and in the retina (42). The intracerebroventricular injection of an AAV9-CBV-*CLN6* vector into postnatal day 1 (P1) *Cln6* ^{nclf} mice reduced the degeneration of photoreceptors in 3-, 6-, and 9-month-old mice compared with untreated controls. In striking contrast, a brain-directed AAV-mediated expression of cathepsin D in a CLN10 mouse model prevented the accumulation of ceroid lipofuscin, the activation of microglia, and neurodegeneration in brain tissues, but not the rapidly progressing retinal degeneration (43). Intravitreal injection of an AAV9 vector encoding *CLN6* into a naturally occurring *CLN6*-deficient South Hampshire sheep model led to a minor reduction of lysosomal storage and retinal atrophy and had no beneficial effects on retinal function as indicated by unaltered ERG amplitudes compared with untreated contralateral eyes (30).

CLN10 DISEASE

The CLN10 mouse deficient in the lysosomal protease cathepsin D (CTSD) is a model for the most severe NCL form, congenital

NCL. Cathepsin D knockout (*Ctsd* ko) mice are characterized by an early-onset loss of photoreceptor cells and a subsequent loss of all other retinal nerve cell types, the accumulation of storage material, lysosomal dysfunction, reduced autophagic flux, reactive astrogliosis and microgliosis, and a shortened lifespan with premature death at P26 (44, 45). The *Ctsd* ko mouse is a valuable model to study the efficacy of experimental ocular therapies due to the early-onset and most rapid progression of retinal degeneration in all NCL forms. Intravitreal administration of an AAVsh10 vector harboring mouse *Ctsd* transduced retinal glial cells and RPE cells (32). Biochemical and morphological analyses of the AAV-treated retinas revealed a restoration of CTSD enzymatic activities close to wild type levels, a complete reduction of lysosomal storage material, the absence of lysosomal hypertrophy, and the preservation of photoreceptor and rod bipolar cells. However, this gene therapy study was unable to clarify whether the retinal function was preserved due to ethical issues related to experiments on severely affected animals at the end stage of the disease.

CLN11 DISEASE

In a mouse model for CLN11 disease, the progranulin knockout (*Pgrn* ko) mouse, autofluorescent storage material accumulation, and the degeneration of photoreceptors and retinal ganglion cells become apparent in 12-month-old mutant mice (46). In a recent study, the therapeutic benefits of intravenous administration of an AAV9.2YF-*Pgrn* vector were compared with the intravitreal delivery of an AAV2.7m8-*Pgrn* vector into *Pgrn* ko mice (33). Systemically administered AAV9 vectors cross the blood-retina- and blood-brain-barriers until 7 days of age. Intravenous delivery of an AAV9.2YF vector encoding murine *PGRN* into P3 or P4 *Pgrn* ko mice led to a reduction of autofluorescent ceroid lipopigments and attenuated the thinning of the outer nuclear layer and the total retina in 12-month-old mutant mice (33). Interestingly, intravitreal injection of an AAV2.7m8-*Pgrn* vector into 1- or 6-month-old *Pgrn* ko mice reduced lipofuscin lipopigments, decreased microglial infiltration, but did not attenuate retinal neurodegeneration. These data suggest that both the route and time of AAV administration are crucial to achieving therapeutic benefits in the retina of *Pgrn* ko mice.

ENZYME REPLACEMENT THERAPY

Ocular enzyme replacement therapies (ERTs) rely on the intravitreal administration of a recombinant soluble lysosomal enzyme and its uptake *via* mannose 6-phosphate receptors, delivery to lysosomes, and cross-correction (47). Therapeutic benefits of ocular ERTs have been tested in animal models for CLN2 and CLN10 diseases (15).

Periodic intravitreal injections of recombinant TPP1 starting at 12 weeks of age into the CLN2 Dachshund dog model led to decreased neurodegeneration in the inner nuclear layer and inhibited declines in ERG amplitudes (48). Intravitreal TPP1 administration also prevented focal retinal detachments in the mutant dogs. A single intravitreal injection of recombinant CTSD into P7 and P14 CLN10 mutant mice partially attenuated lysosomal dysfunction and reduced reactive microgliosis but failed to prevent the photoreceptor loss and retinal degeneration (49). The data suggest that the regular intravitreal administration of a recombinant lysosomal enzyme may be a therapeutic option to treat retinal degeneration and vision loss at least in some NCL forms. A new clinical trial (Clinical Trial gov Identifier: NCT05152914) is currently enrolling patients to test the therapeutic efficacy of intravitreal ERT (Cerliponase alfa) in CLN2 disease.

CELL-BASED THERAPY

Therapeutic benefits of cell-based ERTs were evaluated in NCL animal models (15). Cell transplantation into the retina of NCL models deficient in soluble lysosomal proteins is based on the rationale that the grafted cells secrete the missing lysosomal proteins (donor cells) followed by their internalization *via* mannose 6-phosphate receptors by surrounding defective acceptor cells (47). The lysosomal enzyme-mannose 6-phosphate receptor complexes are internalized and lysosomal enzymes are finally targeted to lysosomes where they are proteolytically activated. Preclinical experiments evaluated the benefits of transplanted stem cells overexpressing the missing lysosomal enzyme. Stem cells transduced with an AAV2-vector carrying the human *PPT1* cDNA were intravitreally implanted at early disease stages into the CLN2 Dachshund model (50). A single injection of these modified stem cells inhibited the pathological changes in retinal morphology and retinal function suggesting that genetically modified stem cells might serve as useful vehicles for a long-term intraocular administration of the soluble lysosomal protein in NCLs. In another approach, neural stem cells that were transduced *ex vivo* with a lentiviral vector harboring murine *Ctsd* cDNA were intravitreally implanted into the CLN10 mouse model of the CLN10 mouse model (32). In treated retinas, the restoration of CTSD enzymatic activities to 44% of wild type levels, a partial decrease of lysosomal storage material, and reduced microgliosis and astrocytosis compared with untreated *Ctsd* knockout retinas were detected. However, the degeneration of different retinal cell types was not prevented by the implanted stem cells. In summary, the data suggest that intravitreal injection of genetically modified stem cells may be an encouraging approach to attenuate retinal degeneration for some NCL forms with soluble lysosomal enzyme deficiencies.

IMMUNOMODULATION

Previous studies showed that the genetic inhibition of the adaptive or innate immune system led to disease-ameliorating effects in the CNS of *Ppt1* ko/*Cln3*$^{\Delta ex1-6}$ ko and *Ppt1* ko mice, respectively (51, 52). In line with these findings, the treatment of *Ppt1* ko or *Cln3*$^{\Delta ex1-6}$ ko mice with immunosuppressive drugs showed therapeutic benefits (53, 54). Oral administration of the immunomodulators fingolimod and teriflunomide prevented retinal thinning in *Ppt1* ko mice and attenuated retinal thinning in *Cln3*$^{\Delta ex1-6}$ ko mice (55). In a genetically modified

Cln3$^{\Delta ex7/8}$ knock-in mouse susceptible to light damage, light exposure resulted in pathological changes, including retinal neurodegeneration, activation of microglia, and accumulation of autofluorescent storage material (56). Treatment of the mutant mice with the antibiotic and anti-inflammatory drug minocycline prior to light stress led to reduced photoreceptor loss and decreased amounts of autofluorescent storage material (56). Based on the strong reactive gliosis present in *Cln6* ^{nclf} retinas, mutant mice were treated with the natural immunomodulators curcumin and docosahexanoic acid [DHA, (57)]. In the curcumin- and DHA-treated *Cln6* ^{nclf} mice, reactive gliosis was attenuated and the decline in visual acuity and ERG amplitudes was delayed when compared with untreated mutant mice.

DISCUSSION

Retinal degeneration and loss of vision are among the major hallmarks of NCLs of NCLs. With the exception of one study on *Cln6* ^{nclf} mice, brain-targeted therapies in NCL animal models had no therapeutic impact on retinal degeneration and loss of retinal function. Therefore, there is an unmet need to design novel eye-targeted therapies. The therapeutic efficacy of eye-targeted experimental therapies in NCL animal models, including gene therapy, enzyme replacement therapy, cell-based therapy, and immunomodulation, were evaluated in the past. Recent experimental ocular gene therapies on animal models with soluble lysosomal enzyme deficiencies (CLN1, CLN5, CLN10, and CLN11) and transmembrane protein deficiencies (CLN3 and CLN6) have shown the strong potential of gene therapeutic approaches to effectively treat NCL-related retinopathies. A major breakthrough in the experimental gene therapy approaches was the identification of the specific cell types that have to be targeted to achieve therapeutic benefit. In the CLN3 and CLN6 mouse models, the AAV-mediated bipolar cell-specific delivery of *CLN3* and *CLN6* was successful in preventing the loss of photoreceptors and bipolar cells, respectively, and to partly preserve retinal function. Furthermore, the intravitreal injection of an AAV9-*CLN5* vector into a CLN5 sheep model largely prevented retinal degeneration and loss of retinal function. Finally, results from a recent study suggest that an AAV-mediated CTSD expression in the retina of a CLN10 mouse model is more potent in preventing retinal degeneration than intravitreal ERT mediated by the injections of recombinant CTSD or by transplantation of neural stem cells overexpressing CTSD. While the results of the eye-targeted therapies are encouraging, most studies have started the treatment prior to the onset of the retinal pathology. Future work thus needs to evaluate whether the treatment strategies are still effective when they are started at the initial or advanced stages of the retinal dystrophy. For clinical applications, gene therapies have the advantage of single dosing compared with enzyme replacement therapies which require repeated administration. Combined therapies targeting the brain and the retina separately may attenuate neurological symptoms and additionally vision loss in patients with NCL. The long-term benefits of these experimental ocular treatment options have to be evaluated in patients with NCL in future clinical studies.

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REFERENCES

1. Mole SE, Cotman SL. Genetics of the neuronal ceroid lipofuscinoses (Batten disease). *Biochim Biophys Acta.* (2015) 1852:2237–41. doi: 10.1016/j.bbadis.2015.05.011
2. Cotman SL, Lefrancois S. CLN3, at the crossroads of endocytic trafficking. *Neurosci Lett.* (2021) 762:136117. doi: 10.1016/j.neulet.2021.136117
3. Wang Y, Zeng W, Lin B, Yao Y, Li C, Hu W, et al. CLN7 is an organellar chloride channel regulating lysosomal function. *Sci Adv.* (2021) 7:eabj9608. doi: 10.1126/sciadv.abj9608
4. Bajaj L, Sharma J, di Ronza A, Zhang P, Eblimit A, Pal R, et al. A CLN6-CLN8 complex recruits lysosomal enzymes at the ER for Golgi transfer. *J Clin Invest.* (2020) 130:4118–32. doi: 10.1172/JCI130955
5. di Ronza A, Bajaj L, Sharma J, Sanagasetti D, Lotfi P, Adamski CJ, et al. CLN8 is an endoplasmic reticulum cargo receptor that regulates lysosome biogenesis. *Nat Cell Biol.* (2018) 20:1370–7. doi: 10.1038/s41556-018-0228-7
6. Williams RE, Mole SE. New nomenclature and classification scheme for the neuronal ceroid lipofuscinoses. *Neurology.* (2012) 79:183–91. doi: 10.1212/WNL.0b013e31825f0547
7. Mole SE, Williams RE, Goebel HH. *The Neuronal Ceroid Lipofuscinoses (Batten disease).* 2nd ed Oxford: Oxford University Press. (2011).
8. Schulz A, Kohlschütter A, Mink J, Simonati A, Williams R, NCL. diseases - clinical perspectives. *Biochim Biophys Acta.* (2013) 1832:1801–6. doi: 10.1016/j.bbadis.2013.04.008
9. Gardner E, Mole SE. The genetic basis of phenotypic heterogeneity in the neuronal ceroid lipofuscinoses. *Front Neurol.* (2021) 12:754045. doi: 10.3389/fneur.2021.754045
10. Specchio N, Ferretti A, Trivisano M, Pietrafusa N, Pepi C, Calabrese C, et al. Neuronal ceroid lipofuscinosis: potential for targeted therapy. *Drugs.* (2021) 81:101–23. doi: 10.1007/s40265-020-01440-7
11. Radke J, Stenzel W, Goebel HH. Human NCL neuropathology. *Biochim Biophys Acta.* (2015) 1852:2262–6. doi: 10.1016/j.bbadis.2015.05.007
12. Ku CA, Hull S, Arno G, Vincent A, Carss K, Kayton R, et al. Detailed clinical phenotype and molecular genetic findings in CLN3-associated isolated retinal degeneration. *JAMA Ophthalmol.* (2017) 135:749–60. doi: 10.1001/jamaophthalmol.2017.1401
13. Khan KN, El-Asrag ME, Ku CA, Holder GE, McKibbin M, Arno G, et al. Specific alleles of CLN7/MFSD8, a protein that localizes to photoreceptor synaptic terminals, cause a spectrum of nonsyndromic retinal dystrophy. *Invest Ophthalmol Vis Sci.* (2017) 58:2906–14. doi: 10.1167/iovs.16-20608

14. Roosing S, van den Born LI, Sangermano R, Banfi S, Koenekoop RK, Zonneveld-Vrieling MN, et al. Mutations in MFSD8, encoding a lysosomal membrane protein, are associated with nonsyndromic autosomal recessive macular dystrophy. *Ophthalmology.* (2015) 122:170–9. doi: 10.1016/j.ophtha.2014.07.040
15. Kohlschütter A, Schulz A, Bartsch U, Storch S. Current and emerging treatment strategies for neuronal ceroid lipofuscinoses. *CNS Drugs.* (2019) 33:315–25. doi: 10.1007/s40263-019-00620-8
16. Schulz A, Ajayi T, Specchio N, de Los Reyes E, Gissen P, Ballon D, et al. Study of intraventricular Cerliponase alfa for CLN2 Disease. *N Engl J Med.* (2018) 378:1898–907. doi: 10.1056/NEJMoa1712649
17. Bond M, Holthaus SM, Tammen I, Tear G, Russell C. Use of model organisms for the study of neuronal ceroid lipofuscinosis. *Biochim Biophys Acta.* (2013) 1832:1842–65. doi: 10.1016/j.bbadis.2013.01.009
18. Shacka JJ. Mouse models of neuronal ceroid lipofuscinoses: useful pre-clinical tools to delineate disease pathophysiology and validate therapeutics. *Brain Res Bull.* (2012) 88:43–57. doi: 10.1016/j.brainresbull.2012.03.003
19. Tang C, Han J, Dalvi S, Manian K, Winschel L, Volland S, et al. A human model of Batten disease shows role of CLN3 in phagocytosis at the photoreceptor-RPE interface. *Commun Biol.* (2021) 4:161. doi: 10.1038/s42003-021-01682-5
20. Wavre-Shapton ST, Calvi AA, Turmaine M, Seabra MC, Cutler DF, Futter CE, et al. Photoreceptor phagosome processing defects and disturbed autophagy in retinal pigment epithelium of $Cln3^{\Delta ex}1$-6 mice modelling juvenile neuronal ceroid lipofuscinosis (Batten disease). *Hum Mol Genet.* (2015) 24:7060–74. doi: 10.1093/hmg/ddv406
21. Liu W, Kleine-Holthaus SM, Herranz-Martin S, Aristorena M, Mole SE, Smith AJ, et al. Experimental gene therapies for the NCLs. *Biochim Biophys Acta Mol Basis Dis.* (2020) 1866:165772. doi: 10.1016/j.bbadis.2020.165772
22. Mole SE, Anderson G, Band HA, Berkovic SF, Cooper JD, Kleine Holthaus SM, et al. Clinical challenges and future therapeutic approaches for neuronal ceroid lipofuscinosis. *Lancet Neurol.* (2019) 18:107–16. doi: 10.1016/S1474-4422(18)30368-5
23. Murray SJ, Mitchell NL. Ocular therapies for neuronal ceroid lipofuscinoses: more than meets the eye. *Neural Regen Res.* (2022) 17:1755–6. doi: 10.4103/1673-5374.332148
24. Johnson TB, White KA, Brudvig JJ, Cain JT, Langin L, Pratt MA, et al. AAV9 gene therapy increases lifespan and treats pathological and behavioral abnormalities in a mouse model of CLN8-Batten Disease. *Mol Ther.* (2021) 29:162–75. doi: 10.1016/j.ymthe.2020.09.033
25. Chen X, Dong T, Hu Y, Shaffo FC, Belur NR, Mazzulli JR, et al. AAV9/MFSD8 gene therapy is effective in preclinical models of neuronal ceroid lipofuscinosis type 7 disease. *J Clin Invest.* (2022) 22:46228. doi: 10.1172/JCI146286
26. Askou AL, Jakobsen TS, Corydon TJ. Retinal gene therapy: an eye-opener of the 21st century. *Gene Ther.* (2021) 28:209–16. doi: 10.1038/s41434-020-0168-2
27. Schön C, Biel M, Michalakis S. Retinal gene delivery by adeno-associated virus (AAV) vectors: strategies and applications. *Eur J Pharm Biopharm.* (2015) 95:343–52. doi: 10.1016/j.ejpb.2015.01.009
28. Griffey M, Macauley SL, Ogilvie JM, Sands MS. AAV2-mediated ocular gene therapy for infantile neuronal ceroid lipofuscinosis. *Mol Ther.* (2005) 12:413–21. doi: 10.1016/j.ymthe.2005.04.018
29. Kleine Holthaus SM, Aristorena M, Maswood R, Semenyuk O, Hoke J, Hare A, et al. Gene therapy targeting the inner retina rescues the retinal phenotype in a mouse model of CLN3 Batten disease. *Hum Gene Ther.* (2020) 31:709–18. doi: 10.1089/hum.2020.038
30. Murray SJ, Russell KN, Melzer TR, Gray SJ, Heap SJ, Palmer DN, 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
31. Kleine Holthaus SM, Ribeiro J, Abelleira-Hervas L, Pearson RA, Duran Y, Georgiadis A, et al. Prevention of photoreceptor cell loss in a Cln6(nclf) mouse model of Batten disease requires CLN6 gene transfer to bipolar cells. *Mol Ther.* (2018) 26:1343–53. doi: 10.1016/j.ymthe.2018.02.027
32. Liu J, Bassal M, Schlichting S, Braren I, Di Spiezio A, Saftig P, et al. Intravitreal gene therapy restores the autophagy-lysosomal pathway and attenuates retinal degeneration in cathepsin D-deficient mice. *Neurobiol Dis.* (2022) 164:105628. doi: 10.1016/j.nbd.2022.105628
33. Zin EA, Han D, Tran J, Morisson-Welch N, Visel M, Kuronen M, et al. Outcomes of progranulin gene therapy in the retina are dependent on time and route of delivery. *Mol Ther Methods Clin Dev.* (2021) 22:40–51. doi: 10.1016/j.omtm.2021.05.009
34. Katz ML, Coates JR, Sibigtroth CM, Taylor JD, Carpentier M, Young WM, et al. Enzyme replacement therapy attenuates disease progression in a canine model of late-infantile neuronal ceroid lipofuscinosis (CLN2 disease). *J Neurosci Res.* (2014) 92:1591–8. doi: 10.1002/jnr.23423
35. Katz ML, Tecedor L, Chen Y, Williamson BG, Lysenko E, Wininger FA, 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) 7:313ra180. doi: 10.1126/scitranslmed.aac6191
36. Whiting REH, Jensen CA, Pearce JW, Gillespie LE, Bristow DE, Katz ML. Intracerebroventricular gene therapy that delays neurological disease progression is associated with selective preservation of retinal ganglion cells in a canine model of CLN2 disease. *Exp Eye Res.* (2016) 146:276–82. doi: 10.1016/j.exer.2016.03.023
37. Cotman SL, Vrbanac V, Lebel LA, Lee RL, Johnson KA, Donahue LR, et al. Cln3(Deltaex7/8) knock-in mice with the common JNCL mutation exhibit progressive neurologic disease that begins before birth. *Hum Mol Genet.* (2002) 11:2709–21. doi: 10.1093/hmg/11.22.2709
38. Staropoli JF, Haliw L, Biswas S, Garrett L, Holter SM, Becker L, et al. Large-scale phenotyping of an accurate genetic mouse model of JNCL identifies novel early pathology outside the central nervous system. *PLoS ONE.* (2012) 7:e38310. doi: 10.1371/journal.pone.0038310
39. Frugier T, Mitchell NL, Tammen I, Houweling PJ, Arthur DG, Kay GW, et al. A new large animal model of CLN5 neuronal ceroid lipofuscinosis in Borderdale sheep is caused by a nucleotide substitution at a consensus splice site (c571 + 1G>A) leading to excision of exon 3. *Neurobiol Dis.* (2008) 29:306–15. doi: 10.1016/j.nbd.2007.09.006
40. Bronson RT, Donahue LR, Johnson KR, Tanner A, Lane PW, Faust JR. Neuronal ceroid lipofuscinosis (nclf), a new disorder of the mouse linked to chromosome 9. *Am J Med Genet.* (1998) 77:289–97.
41. Bartsch U, Galliciotti G, Jofre GF, Jankowiak W, Hagel C, Braulke T. Apoptotic photoreceptor loss and altered expression of lysosomal proteins in the nclf mouse model of neuronal ceroid lipofuscinosis. *Invest Ophthalmol Vis Sci.* (2013) 54:6952–9. doi: 10.1167/iovs.13-12945
42. White KA, Nelvagal HR, Poole TA, Lu B, Johnson TB, Davis S, 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. doi: 10.1016/j.omtm.2020.12.014
43. Shevtsova Z, Garrido M, Weishaupt J, Saftig P, Bahr M, Luhder F, et al. CNS-expressed cathepsin D prevents lymphopenia in a murine model of congenital neuronal ceroid lipofuscinosis. *Am J Pathol.* (2010) 177:271–9. doi: 10.2353/ajpath.2010.091267
44. Koike M, Shibata M, Ohsawa Y, Nakanishi H, Koga T, Kametaka S, et al. Involvement of two different cell death pathways in retinal atrophy of cathepsin D-deficient mice. *Mol Cell Neurosci.* (2003) 22:146–61. doi: 10.1016/S1044-7431(03)00035-6
45. Bassal M, Liu J, Jankowiak W, Saftig P, Bartsch U. Rapid and progressive loss of multiple retinal cell types in cathepsin D-deficient mice-an animal model of CLN10 disease. *Cells.* (2021) 10:696. doi: 10.3390/cells10030696
46. Hafler BP, Klein ZA, Jimmy Zhou Z, Strittmatter SM. Progressive retinal degeneration and accumulation of autofluorescent lipopigments in Progranulin deficient mice. *Brain Res.* (2014) 1588:168–74. doi: 10.1016/j.brainres.2014.09.023
47. Platt FM. d'Azzo A, Davidson BL, Neufeld EF, Tifft CJ. Lysosomal storage diseases. *Nat Rev Dis Primers.* (2018) 4:27. doi: 10.1038/s41572-018-0025-4
48. Whiting REH, Pearce JW, Vansteenkiste DP, Bibi K, Lim S, Robinson Kick G, et al. Intravitreal enzyme replacement preserves retinal structure and function in canine CLN2 neuronal ceroid lipofuscinosis. *Exp Eye Res.* (2020) 197:108130. doi: 10.1016/j.exer.2020.108130
49. Marques ARA, Di Spiezio A, Thiessen N, Schmidt L, Grotzinger J, Lüllmann-Rauch R, et al. Enzyme replacement therapy with recombinant pro-CTSD (cathepsin D) corrects defective proteolysis and autophagy in neuronal ceroid lipofuscinosis. *Autophagy.* (2020) 16:811–25. doi: 10.1080/15548627.2019.1637200

50. Tracy CJ, Whiting RE, Pearce JW, Williamson BG, Vansteenkiste DP, Gillespie LE, et al. Intravitreal implantation of TPP1-transduced stem cells delays retinal degeneration in canine CLN2 neuronal ceroid lipofuscinosis. *Exp Eye Res.* (2016) 152:77–87. doi: 10.1016/j.exer.2016.09.003
51. Groh J, Ribechini E, Stadler D, Schilling T, Lutz MB, Martini R. Sialoadhesin promotes neuroinflammation-related disease progression in two mouse models of CLN disease. *Glia.* (2016) 64:792–809. doi: 10.1002/glia.22962
52. Groh J, Kühl TG, Ip CW, Nelvagal HR, Sri S, Duckett S, et al. Immune cells perturb axons and impair neuronal survival in a mouse model of infantile neuronal ceroid lipofuscinosis. *Brain.* (2013) 136:1083–101. doi: 10.1093/brain/awt020
53. Macauley SL, Wong AM, Shyng C, Augner DP, Dearborn JT, Pearse Y, et al. An anti-neuroinflammatory that targets dysregulated glia enhances the efficacy of CNS-directed gene therapy in murine infantile neuronal ceroid lipofuscinosis. *J Neurosci.* (2014) 34:13077–82. doi: 10.1523/JNEUROSCI.2518-14.2014
54. Seehafer SS, Ramirez-Montealegre D, Wong AM, Chan CH, Castaneda J, Horak M, et al. Immunosuppression alters disease severity in juvenile Batten disease mice. *J Neuroimmunol.* (2011) 230:169–72. doi: 10.1016/j.jneuroim.2010.08.024
55. Groh J, Berve K, Martini R. Fingolimod and teriflunomide attenuate neurodegeneration in mouse models of neuronal ceroid lipofuscinosis. *Mol Ther.* (2017) 25:1889–99. doi: 10.1016/j.ymthe.2017.04.021
56. Dannhausen K, Mohle C, Langmann T. Immunomodulation with minocycline rescues retinal degeneration in juvenile neuronal ceroid lipofuscinosis mice highly susceptible to light damage. *Dis Model Mech.* (2018) 11:dmm033597. doi: 10.1242/dmm.033597
57. Mirza M, Volz C, Karlstetter M, Langiu M, Somogyi A, Ruonala MO, et al. Progressive retinal degeneration and glial activation in the CLN6 (nclf) mouse model of neuronal ceroid lipofuscinosis: a beneficial effect of DHA and curcumin supplementation. *PLoS ONE.* (2013) 8:e75963. doi: 10.1371/annotation/ae907882-62e0-4803-8c00-35b30a649fe9

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Neuronal ceroid lipofuscinosis in the South American-Caribbean region: An epidemiological overview

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Neuronal ceroid lipofuscinoses (NCLs) comprise 13 hereditary neurodegenerative pathologies of very low frequency that affect individuals of all ages around the world. All NCLs share a set of symptoms that are similar to other diseases. The exhaustive collection of data from diverse sources (clinical, genetic, neurology, ophthalmology, etc.) would allow being able in the future to define this group with greater precision for a more efficient diagnostic and therapeutic approach. Despite the large amount of information worldwide, a detailed study of the characteristics of the NCLs in South America and the Caribbean region (SA&C) has not yet been done. Here, we aim to present and analyse the multidisciplinary evidence from all the SA&C with qualitative weighting and biostatistical evaluation of the casuistry. Seventy-one publications from seven countries were reviewed, and data from 261 individuals (including 44 individuals from the Cordoba cohort) were collected. Each NCL disease, as well as phenotypical and genetic data were described and discussed in the whole group. The CLN2, CLN6, and CLN3 disorders are the most frequent in the region. Eighty-seven percent of the individuals were 10 years old or less at the onset of symptoms. Seizures were the most common symptom, both at onset (51%) and throughout the disease course, followed by language (16%), motor (15%), and visual impairments (11%). Although symptoms were similar in all NCLs, some chronological differences could be observed. Sixty DNA variants were described, ranging from single nucleotide variants to large chromosomal deletions. The diagnostic odyssey was probably substantially decreased after medical education activities

promoted by the pharmaceutical industry and parent organizations in some SA&C countries. There is a statistical deviation in the data probably due to the approval of the enzyme replacement therapy for CLN2 disease, which has led to a greater interest among the medical community for the early description of this pathology. As a general conclusion, it became clear in this work that the combined bibliographical/retrospective evaluation approach allowed a general overview of the multidisciplinary components and the epidemiological tendencies of NCLs in the SA&C region.

KEYWORDS

neuronal ceroid lipofuscinoses (NCL), South America-Caribbean, epidemiology, genotype, phenotype

Introduction

Neuronal ceroid lipofuscinoses (NCLs) are rare inherited neurodegenerative disorders of all ages, clinically characterized by progressive loss of speech, vision, cognitive and motor skills, with refractory seizures and early death. Taken together, they are the most common cause of neurodegeneration in childhood (1), although adulthood phenotype has also been described (2). Morphologically, NCLs are characterized by the accumulation of undegraded lipoprotein lipofuscin-like material within lysosomes (1), which includes them in the group of lysosomal storage disorders. To date, thirteen NCL diseases have been described, named according to the affected gene (CLN1-CLN14 diseases, CLN9 disease was suggested and later removed) (3). All the proteins encoded by these genes were defined; however, the specific role of some of them, and how they lead to the lysosomal pathology, remain to be fully elucidated.

Individuals affected by an NCL have been described and studied throughout the world, especially in the Northern Hemisphere where most of the "classical" (CLN1, CLN2, CLN3, CLN4, and CLN10 diseases), as well as the "variant" NCLs (CLN5, CLN6, CLN7, CLN8, CLN11, CLN12, CLN13, and CLN14 diseases), were identified for the first time. For example, CLN5 and CLN8 were first described in Finland (4, 5), CLN6 among the Romany population, and in a big Costa Rican family (6–9), and CLN7 was found in a cohort of Turkish children (10). The information of these individuals was subsequently collected in massive repositories to support the growing number of cases, either specific for NCLs (such as the NCL Resource https://www.ucl.ac.uk/ncl-disease/, supported by the University College London and curated by Dr Sara Mole; and the DEM-CHILD registry, led by Dr Angela Schulz), or for diverse disorders (such as Orphanet https://www.orpha.net/consor/cgi-bin/index.php, and LOVD https://www.lovd.nl). This information is then used, for example, for delineating the natural history of a particular NCL disease, the mutation spectrum, the population level, etc. Later, these results can finally be used as controls or reference points to compare future cases (11–13), and for epidemiological purposes (12).

There is a relative imbalance between the Northern and Southern Hemisphere countries of NCL cases registered in public databases. This might be due to differences in the number of referral centers and research facilities, the development of a robust and efficient national health system, the possibility and time to get a precise diagnosis ("diagnostic odyssey"), the knowledge of the diseases and the registries by the treating physicians, among others. The knowledge of a disease can in turn be increased by some other factors, such as available therapies, the degree of medical education, advocacy activities of family organizations, and other socio-economic factors. Thus, the South American and the Caribbean (hereinafter, SA&C) populations, for example, appear underrepresented in the databases. In the present review, we seek to carry out a multidisciplinary and epidemiological update of the NCL information in SA&C, collecting published information from different medical specialities (neurology, pediatrics, radiology, medical genetics, morphology, enzymology, electrophysiology, etc.) to build a baseline for regional medical use, avoid the registration of repeated cases and find out the regional specificities. Ultimately, we expect to feed regional and international databases and overcome the diagnostic odyssey, misdiagnosis, and underdiagnosis of various countries in this ethnically heterogeneous region (14).

Analysis methodology

A comprehensive bibliographic search was made in the four principal databases of scientific articles (PubMed https://pubmed.ncbi.nlm.nih.gov/, ScienceDirect https://www.sciencedirect.com/, Google Scholar https://scholar.google.com/, and SciELO https://www.scielo.org/), using as keywords "Neuronal ceroid lipofuscinosis," the HGNC approved symbol for each NCL gene (e.g., *PPT1*), its most common alias (e.g.,

CLN1, INCL), and the name of each SA&C country. All articles matching the keywords and including at least one author with a SA&C affiliation were collected. From the first compilation, only those articles with references to affected individuals (such as clinical, biochemical, genetic, morphological studies, etc.) were retrieved and subsequently analyzed. The individuals corresponding to the Cordoba cohort (15), whether or not published, who have a precise diagnosis and complete clinical data, were also included. In these cases, each individual or caregiver signed an informed consent approved by the local ethical board [Inter-institutional Committee of Ethics in Health Research (CIEIS—*Polo Hospitalario*)] authorizing not only the extraction, manipulation, and analysis of their samples for diagnostic purposes but also the dissemination of clinical data ensuring the total anonymity of the individuals. Those subjects with precise references to previous reports were tracked to avoid or reduce the number of duplications. All the information available (clinical, biochemical, genetic, morphological) was collected and analyzed using an Excel datasheet.

Bibliographical evidence

Seventy-one articles published between 1995 and 2022 were collected from the four main literature databases (PubMed, Google Scholar, ScienceDirect, and SciELO), including original articles, reviews, case reports, short communications, and published conference abstracts (a complete list of this bibliography can be found in Supplementary Table 1). It should be noted that an attempt has been made to collect all the information published in public and massive bibliographic portals. Articles published in regional or local journals, conference presentations, or other non-print materials may have been omitted. It is important to highlight the importance of freely sharing clinical cases of rare diseases in public databases for a better understanding of these diseases. The number of publications has had "ups and downs" throughout the period considered with a significant increase in the last decade (Figure 1A). The first publication was by Taratuto et al. (16), in which they presented a group of late infantile NCL (LINCL) and juvenile NCL (JNCL) cases in Argentina. In 2005, a symposium book published by the National University Cordoba (Cordoba, Argentina), and edited by members of the recently formed NCL Program (established at the Children's Hospital of the Province of Cordoba in 2003), conducted an update of the NCL information derived mainly from research groups in SA&C. Individuals from different SA&C countries coursing with any NCL disease were described there, causing the publications peak observed in Figure 1A.

Authors from seven countries (Argentina, Brazil, Chile, Colombia, Mexico, Paraguay, and Venezuela) have collaborated in the publications reviewed (Figure 1B). Moreover, 77% of the articles were led by authors from SA&C. Individuals affected by an NCL were described in Argentina, Brazil, Chile, Colombia, Costa Rica, Mexico, Paraguay, Peru, and Venezuela (Figure 1C). Argentina ($n = 38$ articles) and Brazil ($n = 17$ articles) were the most represented countries, considering both the publications and the individuals described, reflecting the awareness of rare disease studies in the reference centers of these countries in the region.

The SA&C cohort

Phenotypic, genetic, and demographic distribution of cases

Two hundred sixty-one subjects affected by CLN1, CLN2, CLN3, CLN5, CLN6, CLN7, CLN8, CLN11, or CLN12 diseases were described in the literature and incorporated in this review, including some non-published cases of the Cordoba cohort (a complete list of these individuals and their associated medical information is presented in Supplementary Tables 2, 3). In older publications (16–23) the individuals were recorded according to clinical and/or morphological data [presence of intracellular bodies observed by transmission electron microscopy (TEM)], resulting in clinical definitions (e.g., Santavuori-Haltia disease or Jansky-Bielschowsky disease, later redefined as CLN1 and CLN2 diseases, respectively; infantile NCL [INCL], LINCL, JNCL). As genetic testing increased, these clinical definitions were correlated with genetic variants. Thus, individuals with an INCL phenotype were mostly diagnosed with CLN1, LINCL cases mostly with CLN2, and JNCL cases mostly with CLN3 disease. However, the genotype/phenotype correlation is not bidirectional (3). The current nomenclature and classification of NCLs was agreed by an international panel of experts in the clinical, genetic, biological, and morphological fields, and formally established in 2012 (24). It establishes the diagnostic definition of a case taking into account 7 axes (gene, genetic variant, biochemical phenotype, clinical phenotype, ultrastructural phenotype, functionality and other characteristics), which allows a precise definition in light of the heterogeneity of this group of pathologies. Because molecular confirmation was not performed in these old cases, the precise genetic variants affecting these children were not defined, so they were classified in this review as separated entities (NCL, INCL, LINCL, and JNCL in Supplementary Tables 2 , 3).

Ninety-one individuals (35%) of the SA&C cohort are female, 77 are male (29%), and 93 (36%) were not identified. As expected, these values do not differ substantially from the estimated percentages of both genders in SA&C (males: 49.4%, females: 50.6%: Countrymeters https://countrymeters.info/es/South_America,revisedJune26,2022 ; Supplementary Table 3). In addition, 87% of the affected individuals were 10 years old or less at the onset of symptoms (Figure 2). Only those individuals affected by CLN11 or CLN12 disorders were older than 10 years

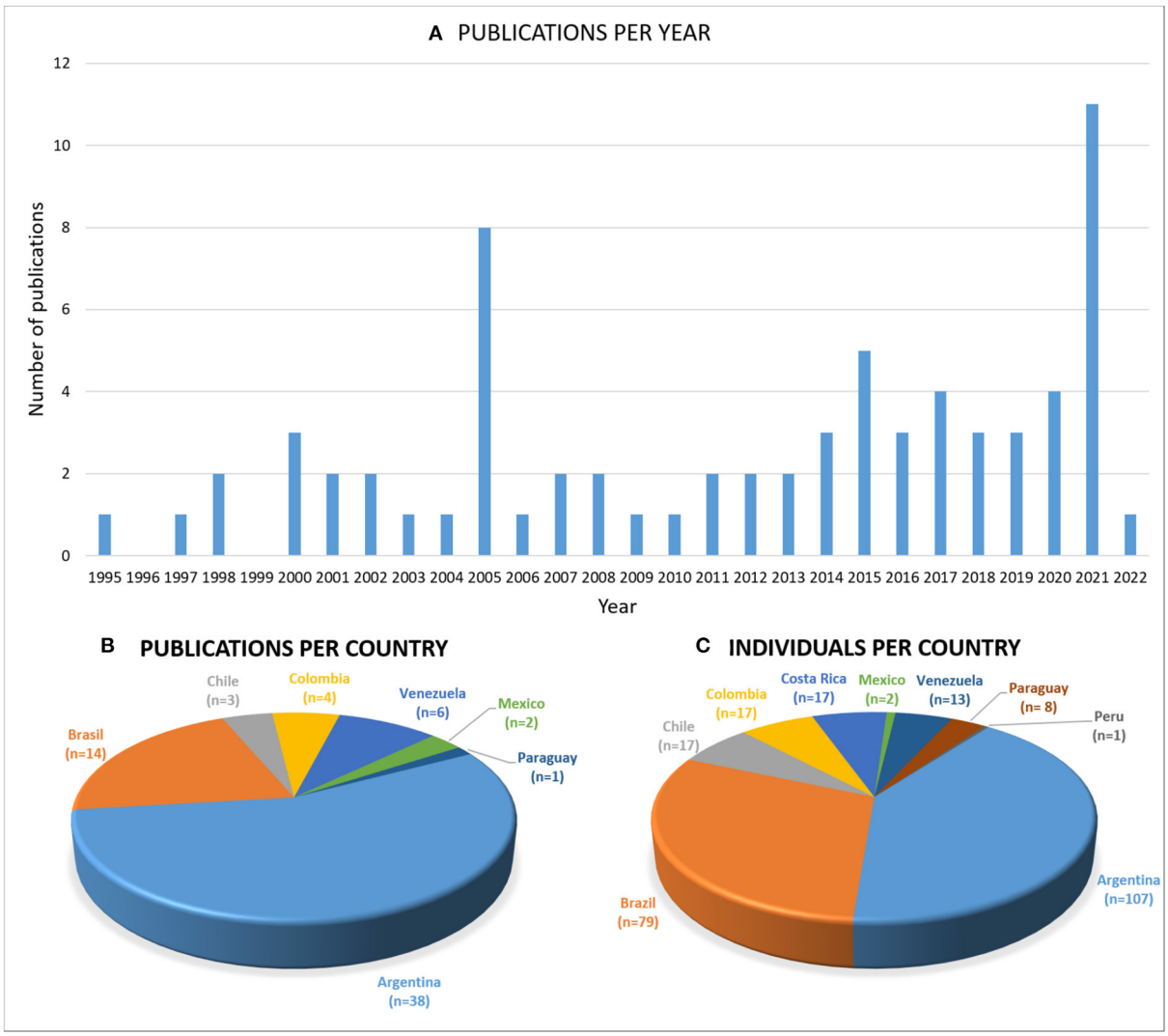
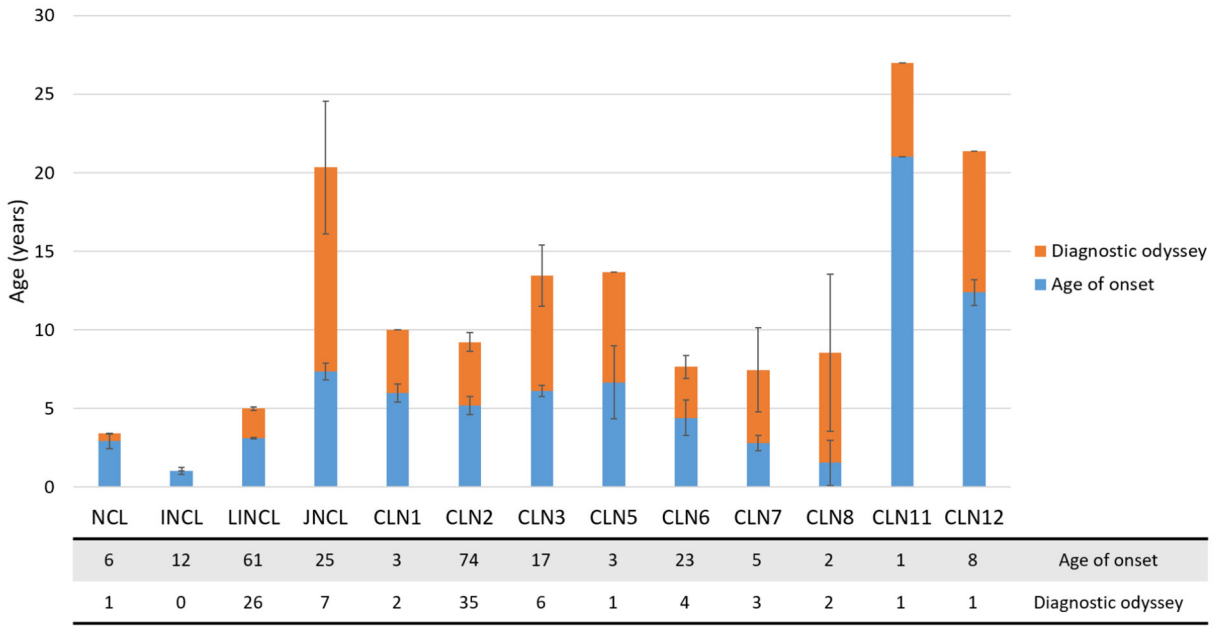


FIGURE 1
The SA&C publications. Graphs showing **(A)** the publications per year and **(B)** per country, and **(C)** the number of individuals described per SA&C country. All types of publications in public databases were collected. The country mentioned in affiliations was taken, even if the author from SA&C was not leading the article. An increasing number of publications (mainly on CLN2) was observed in the last few years. Argentina and Brazil are the most represented regarding the number of publications and individuals described, probably due to the increasing number of professionals and centers specialized in these pathologies.

old at the onset of symptoms. However, one individual affected by CLN12 disease was reported as having developmental delay since birth, although associated with perinatal hypoxia (25).

CLN2 disease is the most represented NCL in the region (n = 91), followed by CLN6 (n = 25) and CLN3 (n = 17). Likewise, LINCL (n = 77) and JNCL (n = 25) were the most abundant phenotypes. Unlike CLN2 cases, which were described along the subcontinent, other variants were observed as local concentrations. For example, an important cluster of CLN6 disease was described in Costa Rica (8, 26), two CLN8 cases in Argentina (27, 28), one CLN11 case in Brazil (29), and one and seven CLN12 cases in Brazil and Chile, respectively (Supplementary Table 2) (25, 30, 31). The CLN11 and CLN12 cases were not directly linked to an NCL. Instead, they were mostly referred to as frontotemporal lobar degeneration (caused by variants in the *GRN/CLN11* gene) or a Parkinson-like neurodegenerative syndrome caused by variants in the *ATP13A2/CLN12* gene. These genetic forms (as well as CLN13 and CLN14) were officially included in the NCL group in 2012, after confirming the presence of lipofuscin-like bodies in the cells of affected individuals (24). However, it is still very common to find them in the literature with the names of their clinical forms, referring to their association with NCLs as an annexe.



	NCL	INCL	LINCL	JNCL	CLN1	CLN2	CLN3	CLN5	CLN6	CLN7	CLN8	CLN11	CLN12
Age of onset	6	12	61	25	3	74	17	3	23	5	2	1	8
Diagnostic odyssey	1	0	26	7	2	35	6	1	4	3	2	1	1

FIGURE 2
Diagnostic odyssey in the SA&C cohort. Bar graph showing the age of onset (mean ± SEM, blue bars) and time to diagnosis (mean ± SEM, orange bars) in the SA&C population analyzed. The age of onset could be recorded for 240 individuals, while the age at diagnosis could only be defined for 89 individuals. The table below the graph indicates de number of individuals included in each analyzed group.

The diagnostic odyssey

The time from the onset of the symptoms to the precise diagnosis did not vary significantly among the confirmed disorders (CLN1-CLN12; Figure 2). The average time to a precise diagnosis was 5.2 ± 1.0 years (mean ± SEM, ranging from 0 to 13 years). Some individuals were diagnosed in a short time because of either a genetic examination or having affected siblings. On the other hand, a prolonged time to diagnosis could be attributed to poor knowledge of these kinds of disorders, particularly before the availability of reliable genetic tests. In some cases (such as in CLN12), the diagnosis was made *post-mortem* (25).

Clinical features

It is widely known that all NCLs share several clinical symptoms, such as seizures, psychomotor decline, and visual failure, which are also present in many other disorders (32). However, the chronological order in which they appear is usually considered at the time of differential diagnosis. The age of onset of different symptoms (seizures, ataxia, motor and cognitive deterioration, behavioral changes, and language and visual failure) was collected and analyzed for the entire SA&C cohort (Figure 3). The most common symptom at onset were seizures (51% of individuals) followed by language disorder (16%), motor impairment (15%), and visual failure (11%; Supplementary Figure 1). It should be noted that many times seizures are the symptom that leads to the first medical consultation, but not the first of the disorder. For example, some cognitive decline could be evident from an early age but attributed to other factors and dismissed. Seizures were also the most common symptom among all NCLs (12/13 groups), followed by pyramidal signs (11/13), cognitive decline (11/13), and language difficulties (10/13). The CLN6 and CLN7 disorders show a very rapid progression of symptoms, with very little variability between cases. Something similar occurs in CLN2 disease, although a greater variability in the onset of swallowing difficulties, behavioral changes and ocular abnormalities has been observed. It should be noted that in this review no discrimination has been made between those known as "classical" and "atypical" phenotypes. Thus, it should be considered that among the CLN2 cases there are "protracted" forms, with a slower symptomatic progression. On the other hand, we have observed a greater chronological dispersion of the symptoms in CLN3 cases (as in JNCL, although we cannot guarantee that they are all from the same genotype). Myoclonus has been observed at later ages in the CLN5, CLN7, CLN8, and CLN12 diseases. Vision loss occurs earlier in the CLN3 and CLN7 disorders. Language difficulties were described as appearing earlier in the CLN2 and CLN6 diseases. Finally, considering the ages of onset of symptoms, the reported cases of CLN1 in the SA&C region could be attributed

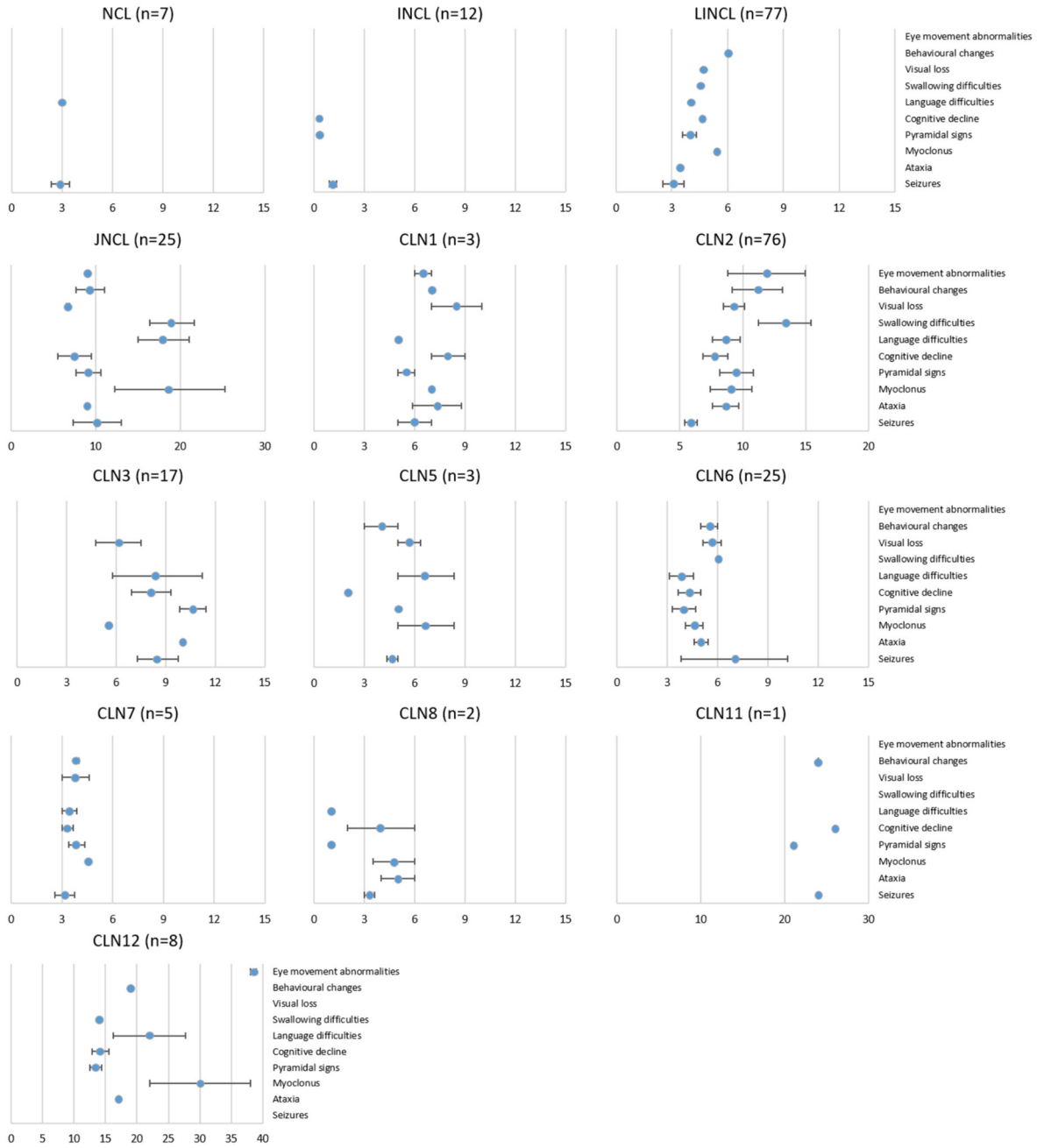


FIGURE 3
Disease evolution of all NCL variants in the SA&C cohort. Graphs showing the age of onset (mean ± SEM) of some symptoms for each NCL genotype and phenotype in the SA&C cohort. Values were obtained directly from clinical records or publications or estimated from the information available in the bibliography. The set of symptoms collected was adapted from Lourenço et al. (11). The number of individuals analyzed for each NCL variant is shown next to the graph titles. Values of the X-axis are expressed in years.

more to a "protracted" than to a "classic" infantile phenotype (Figure 3).

All NCLs are characterized by the presence of lipofuscin-like intralysosomal bodies in all cells. These accumulations are observed by TEM in the form of defined patterns: granular osmiophilic deposits (GROD), curvilinear (CB), fingerprint (FP), or rectilinear bodies (RB), and can occur either alone or mixed (1). For a long time, the examination of tissue biopsies by TEM served as the method for the diagnosis of NCLs, leaving the genetic study for the definition of the particular disease. With the

advent of new generation sequencing technologies, the genetic study is usually suggested within the main tests for diagnosis, reserving the morphological study only for challenging cases. More than half of the individuals in the SA&C cohort were analyzed by TEM ($n = 159$, 59%). The CB pattern was the most abundant among the individuals ($n = 92$, 35%), occurring more frequently in the LINCL ($n = 59$) and CLN2 groups ($n = 20$). On the other hand, the RB pattern has not been observed in the analyzed population (Supplementary Figure 2).

The genetic background of the SA&C cohort

Sixty DNA variants have been described in 119 individuals of the SA&C population (Table 1; Supplementary Figure 3). One individual was reported to have a heterozygous variant in the *CLN6* gene without specifying it (39), and another with a positive linkage analysis pointing to chromosome 15, suggesting a variant in the *CLN6* gene (7). Seventy-seven percent have been variations in the coding sequence of the protein involved, mostly causing missense changes (42%). The intronic changes found ($n = 13$) correspond to splicing regions, having been their pathogenicity confirmed for the most frequent variant in the *TPP1/CLN2* gene, I7 c.887-10A>G, p.Pro295_Gly296insGluAsnPro (40) and the variant I13 c.1306+5G>A, p.Gly398_Leu435del in the *ATP13A2/CLN12* gene (25). Five deletions have been described, spanning few nucleotides [e.g., E9 c.1107_1108delTG, p.Gly370Lysfs*32 in the *TPP1/CLN2* gene (Cordoba cohort)], large intragenic deletions (such as the most frequent variant of CLN3 disease, 1.02 kb deletion, c.462_677del) (36, 41), or large chromosomal deletions (such as the deletion in the 8p23 region including the *CLN8* gene) (28). The pathogenicity of the variants has only been defined in 37% of cases. It should be noted that the pathogenicity of the variants found in the patients described is frequently not defined, or it is only bioinformatically. Experimental validation should be particularly necessary for changes whose effect on the protein is less obvious, such as missense changes or deep intronic variants in splicing regions. Although, on the other hand, the American College of Medical Genetics (ACMG) has proposed guidelines for the interpretation of variants that are currently widely used (42).

When a rare genetic disorder with autosomal recessive inheritance is diagnosed, consanguinity between the parents is usually suspected. The consanguinity could only be confirmed in 10% ($n = 25$) of the population analyzed. However, it could not be defined in more than half of the individuals (57%), suggesting that a higher percentage might be observed (Supplementary Table 3).

Over time, the increase in the number of sequencing performed and their inclusion in the databases lead to an update of the consensus sequences. For example, the update of the human genome sequence toward version GRCh38.p14 (latest to date) was released on February 3, 2022. Although these updates do not usually cause major changes in the nomenclature of the variants described, it would be advisable to include in the publications the data of the version of the database used to identify and validate the variant (for example, the transcript identifier).

Enzymatic tests in the SA&C region

Enzyme activity assays for PPT1 and TPP1 (also for CTSD, although its application is less common) are widely used as a rapid screening method for the CLN1 and CLN2 (and CLN10) diseases, respectively (43–45). However, other NCLs (such as CLN5, CLN6, CLN7, and CLN8) can also show reduced enzymatic values (and not only PPT1 and TPP1 but also all the lysosomal enzymes), thus providing data to guide the diagnosis (27, 46–49). For example, the proteins CLN6 and CLN8, present in the membrane of the endoplasmic reticulum, make up the EGRESS complex responsible for transporting soluble lysosomal enzymes to the Golgi apparatus. It has been shown that the deficiency of any of these proteins causes a decrease in the amount of soluble enzymes that reach the lysosome, thus generating a generalized lysosomal deficiency (46, 49). In the SA&C cohort, only 20% of individuals had PPT1 or TPP1 enzyme activity studied in any of the tissues used [leukocyte pellet, dried blood spot (DBS), or saliva; Supplementary Figure 4]. The most common assay has been the measurement of TPP1 activity in leukocyte pellets (20%) followed by the TPP1 activity assay in DBS (19%). There is a bias in these values toward the analysis of CLN2 disease in these samples. First, enzymatic analysis in leukocyte pellet is considered the "gold standard" for the diagnosis of both CLN1 and CLN2 diseases; therefore, it tends to be more frequently reported in publications (if these tests are performed in several tissues). On the other hand, DBS analysis is the most widespread worldwide, due to the practicality of sending samples over long distances with a minimum of deterioration. PPT1 and TPP1 activity assays in saliva were first described by Kohan et al. in 2005 (43). Despite being minimally invasive and with quantitative robustness like that obtained with the leukocyte pellet (50), its use has not reached the extent of other samples. Lastly, enzyme assays are not always the first option for NCL screening. When performing a genetic test and observing DNA variants in genes other than *PPT1/CLN1* or *TPP1/CLN2* (or *CTSD/CLN10*), enzymatic assays are not performed, and quite sensibly. In addition, results "within the reference interval" (i.e., within the range of control values) may be usually disregarded for final publication.

TABLE 1 List of DNA variants described in the SA&C cohort.

Gene	Variant	Total alleles	Allelic frequency	Publications
CLN1	E5 c.451C>T, p.Arg151* (P)	1	0.0019	Cordoba cohort
	I3 c.363-3T>G, p.? (U)	1	0.0019	Cordoba cohort
CLN2	E11 c.1424C>T, p.Ser475Leu (PP)	4	0.0077	Cordoba cohort, (11)
	E4 c.311T>A, p.Leu104* (P)	3	0.0057	Cordoba cohort
	I2 c.89+5G>C, p.? (U)	2	0.0038	Cordoba cohort
	E8 c.1048C>T, p.Arg350Trp (PP)	8	0.0153	Cordoba cohort, (11)
	I7 c.887-10A>G, p.Pro295_Gly296insGluAsnPro (P)	20	0.0383	Cordoba cohort, (11, 33)
	E7 c.827A>T, p.Asp276Val (P)	21	0.0402	Cordoba cohort, (11)
	I1 c.17+3G>T, p.? (U)	1	0.0019	Cordoba cohort
	E6 c.622C>T, p.Arg208* (P)	11	0.0211	Cordoba cohort, (11, 33)
	E8 c.1016G>A, p.Arg339Gln (PP)	2	0.0038	Cordoba cohort
	E3 c.196C>T, p.Gln66* (P)	5	0.0096	Cordoba cohort, (11)
	E11 c.1340G>A, p.Arg447His (PP)	6	0.0115	Cordoba cohort, (11, 34)
	E7 c.225G>A, p.Gln75Gln (P)	1	0.0019	(35)
	E11 c.1266G>C, p.Gln422His (PP)	1	0.0019	(11)
	E12 c.1438G>A, p.Val480Met (U)	6	0.0115	(11, 33)
	I8 c.1076-2A>T, p.? (PP)	10	0.0192	(11, 33)
	E11 c.1358C>T, p.Ala453Val (PP)	1	0.0019	Cordoba cohort
	E11 c.1358C>A, p.Ala453Asp (PP)	2	0.0038	Cordoba cohort
	E13 c.1603G>C, p.Gly535Arg (PP)	4	0.0077	Cordoba cohort, (11)
	E9 c.1107_1108delTG, p.Gly370Lysfs*32 (PP)	1	0.0019	Cordoba cohort
	I5 c.509-1G>C, p.? (PP)	1	0.0019	Cordoba cohort
	I12 c.1552-1G>A, p.? (PP)	2	0.0038	(11, 34)
	E6 c.503_504insTGGA, p.Phe169Glyfs*20 (P)	1	0.0019	(35)
	E12 c.1439T>G, p.Val480Gly (PP)	1	0.0019	(11)
	E11 c.1343C>A, p.Ala448Asp (PP)	1	0.0019	(11)
	E6 c.616C>T, p.Arg206Cys (P)	2	0.0038	(33)
	E5 c.471C>A, p.Tyr157* (PP)	1	0.0019	(33)
CLN3	E14 c.1195G>T, p.Glu399* (PP)	1	0.0019	Cordoba cohort
	1.02 kb deletion (c.462_677del)	10	0.0192	Cordoba cohort, (36)
	E6 c.400T>C, p.Cys134Arg (PP)	1	0.0019	Cordoba cohort
	E13 c.1000C>T, p.Arg334Cys (PP)	1	0.0019	Cordoba cohort
CLN5	E1 c.291_292insC, p.Ser98Leufs*13 (PP)	2	0.0038	Cordoba cohort
	E2 c.335G>A, p.Arg112His (P)	4	0.0077	(37)
CLN6	I4 c.486+8C>T, p.? (U)	1	0.0019	Cordoba cohort
	E4 c.307C>T, p.Arg103Trp (PP)	1	0.0019	Cordoba cohort
	E4 c.461_463delTCA, p.Ile153del (P)	1	0.0019	Cordoba cohort
	E6 c.662A>C, p.Tyr221Ser (PP)	2	0.0038	(8)
	E3 c.214G>T, p.Glu72* (P)	23	0.0441	(8, 26)
	E6 c.552_552delC, p.Phe185Serfs*21 (PP)	2	0.0038	(38)
	E5 c.510_512delCTA, p.Tyr171del (PP)	2	0.0038	(8)
	E7 c.755G>A, p.Arg252His (PP)	1	0.0019	Cordoba cohort
	E6 c.555_556insC, p.Phe186Leufs*16 (PP)	1	0.0019	Cordoba cohort
	E3 c.250T>A, p.Tyr84Asn (PP)	1	0.0019	Cordoba cohort
	E4 c.368G>A, p.Gly123Asp (P)	7	0.0134	(8)
	E7 c.722T>C, p.Met241Thr (P)	7	0.0134	(8)
	E3 c.244G>C, p.Gly82Arg (PP)	2	0.0038	(38)
	I2 c.198+104T>C (U)	16	0.0307	(26)

(Continued)

TABLE 1 Continued

Gene	Variant	Total alleles	Allelic frequency	Publications
CLN7	I2 c.63-4delC, p.? (U)	2	0.0038	Cordoba cohort
	E3 c.103C>T, p.Arg35* (P)	5	0.0096	Cordoba cohort
	E12 c.1444C>T, p.Arg482* (P)	1	0.0019	Cordoba cohort
	I9 c.863+1G>A, p.? (PP)	1	0.0019	Cordoba cohort
CLN8	E2 c.1A>G, p.? (PP)	1	0.0019	Cordoba cohort
	Deletion of 378.6 kb in 8p23 region	2	0.0038	(28)
	E3 c.792C>G, p.Asn264Lys (P)	1	0.0019	Cordoba cohort
CLN11	E8 c.767_768insCC, p.Gln257Profs*27 (P)	2	0.0038	(29)
CLN12	E15 c.1510G>C, p.Gly504Arg (PP)	2	0.0038	(30)
	I22 c.2529+1G>A, p.? (P)	2	0.0038	(31)
	E26 c.3057_3057delC, p.Tyr1020Thrfs*3 (P)	7	0.0134	(25, 31)
	I13 c.1306+5G>A, p.Gly398_Leu435del (P)	5	0.0096	(25)

The allelic frequency was calculated based on all the individuals in the SA&C cohort. P, pathogenic; PP, probably pathogenic; U, uncertain significance.

Neuroimaging and electrophysiology studies

Certain features common to all NCLs were noted in the results of neurophysiological and imaging studies. Magnetic resonance images and computed tomography studies showed, to a greater or lesser extent, some degree of cerebral and/or cerebellar atrophy, with signal hyperintensity in periventricular regions also being very common. Electroencephalography studies generally showed slowing of basal rhythms, with focal or generalized epileptic paroxysms, with generalized polyspike or spike-wave phenomena. Electroretinogram and visual evoked potentials studies were performed mostly in those cases showing some degree of visual loss, observing optic nerve atrophy, retinitis pigmentosa, pale pupils, and thinning of retinal capillaries, among others. Supplementary Table 3 shows detailed information on the results of the studies performed on all patients in the SA&C cohort.

Concluding remarks

Neuronal ceroid lipofuscinoses are a heterogeneous group of rare disorders sharing a handful of symptoms that, in turn, are common in other neurodegenerative pathologies. However, particular symptoms, as well as the sequential combination of them, can be recognized in NCLs, helping in some way to guide the diagnosis. To improve this, it is important to collect and study the set and sequence of phenotypic features of each precisely diagnosed NCL through its manifestation in each individual. The study of the SA&C population of affected individuals is in this sense a "black pearl" to delineate the clinical assessment of new cases. Although many subjects have been reported as coursing a "classical" natural history, many others have broken the "classical" forms introducing "atypical" symptoms or disease evolution to the spectrum of NCL phenotypes. Such are the cases of CLN2 (the "atypical" or "protracted" variant described in the Cordoba cohort) (51), CLN6 (Costa Rica's variant) (8, 26, 52, 53), and CLN8 diseases (the congenital variant) (27). This heterogeneity may be due to the ethnic and genetic diversity imprinted on the SA&C population, as suggested by some authors (50).

This review brings with it a series of limitations: the literature included was only that available in public databases; many relevant clinical data have not been reported in the publications, either due to omission, ignorance or were simply out of the scope of the work; the criteria for defining a non-obvious symptom may vary between different clinicians, leading sometimes to a late description of its onset: despite our efforts, some individuals were likely counted more than once in our analysis due to inefficient identification in the literature; and on the other hand, those cases that have not been published have been left out of this work (except for the cases of our research center). However, the complexity and quantity of the information collected allow us to address some points: (1) the multidisciplinary approach allowed us to describe and compare the evolution of each NCL in the region and to recognize some of the peculiarities of each genotype. Despite the phenotypic similarities between NCLs with each other and with other pathologies, there are certain variations (mainly chronological) that may guide medical diagnosis. Similarly, a multidisciplinary study (clinical, genetic, enzymatic, radiological, ophthalmological, etc.) of each particular case is always necessary; (2) Certain NCLs are more studied worldwide than others, such as CLN2 and CLN3. In principle, this may be since they are the most abundant NCLs, and therefore, the most important for the prompt search for effective treatments. However, the commercial availability of enzyme replacement

therapy for CLN2 in 2017 has aroused medical interest in the early diagnosis of this pathology, and scientific interest in studying the results of its application. This led to an increase in published articles on this pathology, both worldwide and in SA&C; (3) The less prevalent phenotypes may still be underdiagnosed in many countries. Medical and technological advances promote awareness of some diseases, as happened with therapy for CLN2. This can pose two future scenarios: that the search for a "better known" disease leads to the diagnosis of another "less known," or that the "less known" are underdiagnosed. Despite this, since NCLs are still little known to many health professionals, underdiagnosis may be generalized for all of them; (4) Knowledge about these rare diseases was increased in countries such as Argentina, Brazil, and Chile as an indicator of the impact of genomic technology, new therapeutic interventions based on enzyme replacement technology and gene therapy, medical education, and family advocacy; (5) The diagnostic odyssey gradually decreased (mainly in the most advanced countries of the region), probably as the diseases became better known by the local medical community after the appearance of new therapeutic solutions on the immediate horizon, as well as the earlier implementation of specific (panels of genes) or generalized genetic studies (genomic or exomic studies); (6) Diagnosis through TEM has been gradually replaced by genetic studies. Currently, the wide availability of genetic tests, as well as the minimal intervention on the patient (blood sample vs. tissue biopsy) has promoted this transition. However, since the accumulation of intralysosomal compounds is the pathognomonic feature of NCLs, this practice is suggested in cases where the clinic and genetics do not allow arriving at the same diagnosis.

In summary, an exhaustive search of the public literature on NCLs by SA&C authors, as well as referring to affected individuals in the same region, has been performed for this review. In the same way, the clinical information of 44 individuals included in the Cordoba cohort since 2003 has been compiled. Altogether, 71 scientific articles and 261 individuals affected by any NCL have been analyzed, becoming the largest compilation to date of clinical and bibliographic information on NCLs for SA&C. This work aims to promote the creation and/or improvement of public databases for the region, strengthen the information network on NCLs, lay the foundations for rigorous criteria for clinical data collection and help diagnose these challenging pathologies.

Author contributions

GG contributed to clinical assessment, data collection, writing, and review. ACV contributed to data collection and review. IAC contributed to the planning and revision of the work. AB and NG contributed to the clinical assessment. JCV and EAF contributed to bioinformatics and review. ADP contributed to the review. IN contributed to data collection, the planning, and revision of the work. FP contributed to planning, data collection and analysis, writing, graphical formatting, and review. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.920421/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Onset symptoms in the SA&C cohort. Graph showing the symptoms (or set of them) and percentage of total individuals that showed them at the onset of the disorder. In the cases of individuals that showed more than one symptom at onset, they were added to each group. Seizures are significantly the most common symptom at onset (possibly overestimated) followed by language disorders, motor impairment and visual failure. The NA group represents those individuals with data not available.

SUPPLEMENTARY FIGURE 2
Lipofuscin-like accumulation in the SA&C cohort of individuals. Bar graph showing the percentage of individuals affected by each NCL disorder that showed any kind of lipofuscin-like accumulation observed by TEM. The total number of individuals in each NCL disease is shown on the X-axis. Curvilinear bodies (CB) are significantly the most represented pattern observed in most of the NCLs, followed by granular osmiophilic deposits (GROD) and fingerprints (FP). Rectilinear bodies (RB) were not observed in any of the individuals analyzed. Those individuals that showed more than one pattern (mixed) were added to all the corresponding groups.

SUPPLEMENTARY FIGURE 3
Summary of the DNA variants information in the SA&C cohort. Graphs showing information about the **(A)** position, **(B)** protein effect and **(C)** predicted consequence of all DNA variants described in the SA&C cohort. In those cases where the pathogenicity of the DNA variant was not defined in the publication, it was predicted bioinformatically by using Mutation Taster (https://www.mutationtaster.org/).

SUPPLEMENTARY FIGURE 4
Enzymatic analyzes in SA&C. Bar graphs showing the number of individuals analyzed enzymatically for each type of sample and NCL disorder. TPP1 was significantly more analyzed than PPT1 in all samples and NCLs. In addition, leukocytes and dried blood spots (DBS) are significantly more used than saliva. In turn, it is observed that the largest number of tests were performed for individuals affected by CLN2 disease, as expected. Likely, there is a bias mainly toward CLN2 disease on the total number of tests performed, due to the lack of information on enzyme assays in other NCLs. N, the total number of individuals analyzed for each NCL disorder. If an individual was analyzed for more than one tissue and/or enzyme, it was added to all the corresponding groups.

References

1. Haltia M. The neuronal ceroid-lipofuscinoses. *J Neuropathol Exp Neurol.* (2003) 62:1–13. doi: 10.1093/jnen/62.1.1

2. Berkovic SF, Staropoli JF, Carpenter S, Oliver KL, Kmoch S, Anderson GW, et al. Diagnosis and misdiagnosis of adult neuronal ceroid lipofuscinosis (Kufs disease). *Neurology.* (2016) 87:579–84. doi: 10.1212/WNL.0000000000002943

3. Gardner E, Mole SE. The genetic basis of phenotypic heterogeneity in the neuronal ceroid lipofuscinoses. *Front Neurol.* (2021) 12:754045. doi: 10.3389/fneur.2021.754045

4. Hirvasniemi A, Lang H, Lehesjoki AE, Leisti J. Northern epilepsy syndrome: an inherited childhood onset epilepsy with associated mental deterioration. *J Med Genet.* (1994) 31:177–82. doi: 10.1136/jmg.31.3.177

5. Williams R, Santavuori P, Peltonen L, Gardiner RM, Jrvelä I. A variant form of late infantile neuronal ceroid lipofuscinosis (CLN5) is not an allelic form of batten (Spielmeyer-Vogt-Sjögren, CLN3) disease: exclusion of linkage to the CLN3 region of chromosome 16. *Genomics.* (1994) 20:289–90. doi: 10.1006/GENO.1994.1168

6. Lake BD, Cavanagh NPC. Early-juvenile batten's disease–a recognisable sub-group distinct from other forms of Batten's disease. Analysis of 5 patients. *J Neurol Sci.* (1978) 36:265–71. doi: 10.1016/0022-510X(78)90087-4

7. Peña JA, Cardozo JJ, Montiel CM, Molina OM, Boustany RM. Serial MRI findings in the costa rican variant of neuronal ceroid-lipofuscinosis. *Pediatric Neurol.* (2001) 25:78–80. doi: 10.1016/S0887-8994(01)00284-3

8. Sharp JD, Wheeler RB, Parker KA, Gardiner MR, Williams RE, Mole SE. Spectrum of CLN6 mutations in variant late infantile neuronal ceroid lipofuscinosis. *Hum Mutat.* (2003) 22:35–42. doi: 10.1002/humu.10227

9. Elleder M, Franc J, Kraus J, Nevšímalová S, Sixtová K, Zeman J. Neuronal ceroid lipofuscinosis in the Czech Republic: analysis of 57 cases. Report of the "Prague NCL group." *Eur J Paediatr Neurol.* (1997) 1:109–14. doi: 10.1016/S1090-3798(97)80041-4

10. Wheeler RB, Sharp JD, Mitchell W, Bate SL, Williams RE, Lake BD, et al. A new locus for variant late infantile neuronal ceroid lipofuscinosis-CLN7. *Mol Genet Metab.* (1999) 66:337–8. doi: 10.1006/mgme.1999.2804

11. Lourenço CM, Pessoa A, Mendes CC, Rivera-Nieto C, Vergara D, Troncoso M, et al. Revealing the clinical phenotype of atypical neuronal ceroid lipofuscinosis type 2 disease: Insights from the largest cohort in the world. *J Paediatr Child Health.* (2020) 57:519–25. doi: 10.1111/jpc.15250

12. Nickel M, Simonati A, Jacoby D, Lezius S, Kilian D, van de Graaf B, et al. Disease characteristics and progression in patients with late-infantile neuronal ceroid lipofuscinosis type 2 (CLN2) disease: an observational cohort study. *Lancet Child Adol Health.* (2018) 2:582–90. doi: 10.1016/S2352-4642(18)30179-2

13. Nickel M, Schulz A. Natural history studies in NCL and their expanding role in drug development: experiences from CLN2 disease and relevance for clinical trials. *Front Neurol.* (2022) 13:785841. doi: 10.3389/FNEUR.2022.785841

14. Cismondi IA, Kohan R, Adams HR, Bond M, Brown R, Cooper JD, et al. Guidelines for incorporating scientific knowledge and practice on rare diseases into higher education: neuronal ceroid lipofuscinoses as a model disorder. *Biochim Biophys Acta.* (2015) 1852:2316–23. doi: 10.1016/j.bbadis.2015.06.018

15. Pesaola F, Guelbert G, Venier AC, Cismondi IA, Becerra A, Vazquez JCG, et al. "Atypical" phenotypes of neuronal ceroid lipofuscinosis: the argentine experience in the genomic era. *J Inborn Errors Metab Screen.* (2021) 9:e20210009. doi: 10.1590/2326-4594-jiems-2021-0009

16. Taratuto AL, Saccoliti M, Sevlever G, Ruggieri VL, Arroyo H, Herrero M, et al. Childhood neuronal ceroid-lipofuscinoses in Argentina. *Am J Med Genet.* (1995) 57:144–9. doi: 10.1002/ajmg.1320570207

17. Torres LFB, Jacob GVV, de Noronha L, Sampaio GA, Antoniuk S, Bruck I. Estudo por microscopia eletrônica em doenças neurodegenerativas na infância. *Arquiv Neuro Psiquiatria.* (1997) 55:788–94. doi: 10.1590/S0004-282X1997000500016

18. Peña JA, Montiel-Nava C, Delgado W, Hernández ML, Cardozo JJ, Mora E, et al. Characterization of neuronal ceroid lipofuscinosis in venezuelan children. *Rev Neurol.* (2004) 38:42–8. doi: 10.33588/rn.3801.2003300

19. Puga ACS, Jardim LB, Chimelli L, de Souza CFM, Clivati M. Neuronal ceroid lipofuscinoses: a clinical and morphological study of 17 patients from Southern Brazil. *Arquiv Neuro Psiquiatria.* (2000) 58:597–606. doi: 10.1590/S0004-282X2000000400001

20. Quagliato EMAB, Rocha DM, Sacai PY, Watanabe SS, Salomão SR, Berezovsky A. Retinal function in patients with the neuronal ceroid lipofuscinosis phenotype. *Arquiv Brasil Oftalmol.* (2017) 80:215–9. doi: 10.5935/0004-2749.20170053

21. Rosemberg S. Diagnosis of metabolic diseases of the nervous system in children through ultrastructural analysis of non cerebral tissue. *Arquiv Neuro Psiquiatria.* (1998) 56:436–42. doi: 10.1590/s0004-282x1998000300013

22. Vasques CO, Valério RMF, Reed UC, Grossman RM, Kok F. Pitfalls in the clinical and electroencephalographic diagnosis of ceroid lipofuscinosis. *Arquiv Neuro Psiquiatr.* (2005) 63:93–6. doi: 10.1590/s0004-282x2005000100017

23. Gama RL, Nakayama M, Távora DGF, Alvim TCDL, Nogueira CD, Portugal D. Neuronal ceroid lipofuscinosis: clinical and neuroradiological findings. *Arquiv Neuro Psiquiatr.* (2007) 65:320–6. doi: 10.1590/s0004-282x2007000200025

24. Williams RE, Mole SE. New nomenclature and classification scheme for the neuronal ceroid lipofuscinoses. *Neurology.* (2012) 79:183–91. doi: 10.1212/WNL.0b013e31825f0547

25. Behrens MI, Brüggemann N, Chana P, Venegas P, Kägi M, Parrao T, et al. Clinical spectrum of Kufor-Rakeb syndrome in the chilean kindred with ATP13A2 mutations. *Mov Disord.* (2010) 25:1929–37. doi: 10.1002/MDS.22996

26. Badilla-Porras R, Echeverri-McCandless A, Weimer JM, Ulate-Campos A, Soto-Rodríguez A, Gutiérrez-Mata A, et al. Neuronal ceroid lipofuscinosis type 6

(CLN6) clinical findings and molecular diagnosis: costa rica's experience. *Orphanet J Rare Dis.* (2022) 17:13. doi: 10.1186/S13023-021-02162-Z

27. Pesaola F, Kohan R, Cismondi IA, Guelbert N, Pons P, Oller-Ramirez AM, et al. Congenital CLN8 disease of neuronal ceroid lipofuscinosis: a novel phenotype. *Rev Neurol.* (2019) 68:155–9. doi: 10.33588/rn.6804.2018217

28. Beesley C, Guerreiro RJ, Bras JT, Williams RE, Taratuto AL, Eltze C, et al. CLN8 disease caused by large genomic deletions. *Mol Genet Genomic Med.* (2017) 5:85–91. doi: 10.1002/mgg3.263

29. Faber I, Prota JRM, Martinez ARM, Lopes-Cendes I, França MC. A new phenotype associated with homozygous GRN mutations: complicated spastic paraplegia. *Eur J Neurol.* (2017) 24:e3–4. doi: 10.1111/ene.13194

30. di Fonzo A, Chien HF, Socal M, Giraudo S, Tassorelli C, Iliceto G, et al. ATP13A2 missense mutations in juvenile parkinsonism and young onset Parkinson disease. *Neurology.* (2007) 68:1557–62. doi: 10.1212/01.WNL.0000260963.08711.08

31. Miranda M, Harmuth F, Bustamante ML, Rossi M, Sturm M, Magnusson ÓT, et al. Intermediate phenotype of ATP13A2 mutation in two chilean siblings: towards a continuum between parkinsonism and hereditary spastic paraplegia. *Parkinsonism Relat Disord.* (2020) 81:45–7. doi: 10.1016/J.PARKRELDIS.2020.10.004

32. Simonati A, Williams RE. Neuronal ceroid lipofuscinosis: the multifaceted approach to the clinical issues, an overview. *Front Neurol.* (2022) 13:811686. doi: 10.3389/FNEUR.2022.811686

33. Espitia Segura OM, Hernández Z, Mancilla NI, Naranjo RA, Tavera L. Real world effectiveness of cerliponase alfa in classical and atypical patients. A case series. *Mol Genet Metab Rep.* (2021) 27:100718. doi: 10.1016/J.YMGMR.2021.100718

34. Curiati Mendes CS, Rand M, Curiati MA, Martins AM. Atypical neuronal ceroid lipofuscinosis type 2 (CLN2 disease): a case report. WORLD Symposium™ 2020. *Mol Genet Metab.* (2020) 129:1–184. doi: 10.1016/j.ymgme.2019.11.094

35. Nunes A, Meira J, Cunha C, Veiga M, Magalhães APS de, Málaga DR, et al. A case report on the challenging diagnosis of neuronal ceroid lipofuscinosis type 2 (CLN2). *J Inborn Errors Metab Screen.* (2020) 8:e20200010. doi: 10.1590/2326-4594-jiems-2020-0010

36. Ortiz J, Lezcano M, Aldana A, Paredes M, Morel Z, Esquivel I, et al. Lipofuscinosis ceroidea neuronal infantil tardía (Jansky- Bielchowsky). Estudio de casos. *Pediatría.* (2014) 41:33–44.

37. Pineda-Trujillo N, Cornejo W, Carrizosa J, Wheeler RB, Múnera S, Valencia A, et al. A CLN5 mutation causing an atypical neuronal ceroid lipofuscinosis of juvenile onset. *Neurology.* (2005) 64:740–742. doi: 10.1212/01.WNL.0000151974.44980.F1

38. Bravo Oro A, Saavedra Alanís VM, Reyes Vaca JG, Espinosa Tanguma R, Shiguetomi Medina JM, Esmer C. Neuronal ceroid lipofuscinosis. Type 6 late infantile variant in two compound heterozygous siblings with novel mutations. *Rev Neurol.* (2021) 73:368. doi: 10.33588/RN.7310.2021174

39. González Pabón DV, Bermejo Padilla SM, Espinosa García E. Lipofuscinosis ceroidea neuronal 6 (enfermedad Kufs tipo A): Reporte de caso en Colombia. *Acta Neurol Colomb.* (2021) 37:197–202. doi: 10.22379/24224022388

40. Bessa CJP, Teixeira CA, Dias A, Alves M, Rocha S, Lacerda L, et al. CLN2/TPP1 deficiency: the novel mutation IVS7-10A>G causes intron retention and is associated with a mild disease phenotype. *Mol Genet Metab.* (2008) 93:66–73. doi: 10.1016/j.ymgme.2007.08.124

41. Valadares ER, Pizarro MX, Oliveira LR, Amorim RHC de, Pinheiro TMM, Grieben U, et al. Juvenile neuronal ceroid-lipofuscinosis: clinical and molecular investigation in a large family in Brazil. *Arq Neuropsiquiatr.* (2011) 69:13–8. doi: 10.1590/s0004-282x2011000100004

42. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. *Genet Med.* (2015) 17:405–24. doi: 10.1038/gim.2015.30

43. Kohan R, Noher de Halac I, Tapia Anzolini V, Cismondi IA, Oller-Ramirez AM, Paschini Capra A, et al. Palmitoyl protein thioesterase1 (PPT1) and tripeptidyl peptidase-I (TPP-I) are expressed in the human saliva. A reliable and non-invasive source for the diagnosis of infantile (CLN1) and late infantile (CLN2) neuronal ceroid lipofuscinoses. *Clin Biochem.* (2005) 38:492–4. doi: 10.1016/j.clinbiochem.2004.12.007

44. Lukacs Z, Santavuori P, Keil A, Steinfeld R, Kohlschütter A. Rapid and simple assay for the determination of tripeptidyl peptidase and palmitoyl protein thioesterase activities in dried blood spots. *Clin Chem.* (2003) 49:509–11. doi: 10.1373/49.3.509

45. van Diggelen OP, Keulemans JL, Kleijer WJ, Thobois S, Tilikete C, Voznyi YV. Pre- and postnatal enzyme analysis for infantile, late infantile and adult neuronal ceroid lipofuscinosis (CLN1 and CLN2). *Eur J Paediatr Neurol.* (2001) 5(Supple. A):189–92. doi: 10.1053/ejpn.2001.0509

46. di Ronza A, Bajaj L, Sharma J, Sanagasetti D, Lotfi P, Adamski CJ, et al. CLN8 is an endoplasmic reticulum cargo receptor that regulates lysosome biogenesis. *Nat Cell Biol.* (2018) 20:1370–7. doi: 10.1038/s41556-018-0228-7

47. Yap SQ, Kim WD, Huber RJ. Mfsd8 modulates growth and the early stages of multicellular development in dictyostelium discoideum. *Front Cell Dev Biol.* (2022) 10:930235. doi: 10.3389/FCELL.2022.930235

48. Basak I, Hansen RA, Ward ME, Hughes SM. Deficiency of the lysosomal protein CLN5 alters lysosomal function and movement. *Biomolecules.* (2021) 11:1412. doi: 10.3390/BIOM11101412

49. Bajaj L, Sharma J, di Ronza A, Zhang P, Eblimit A, Pal R, et al. A CLN6-CLN8 complex recruits lysosomal enzymes at the ER for golgi transfer. *J Clin Invest.* (2020) 130:4118–32. doi: 10.1172/jci130955

50. Kohan R, Pesaola F, Guelbert N, Pons P, Oller-Ramirez AM, Rautenberg G, et al. The neuronal ceroid lipofuscinoses program: a translational research experience in Argentina. *Biochim Biophys Acta.* (2015) 1852:2300–11. doi: 10.1016/j.bbadis.2015.05.003

51. Kohan R, Carabelos MN, Xin W, Sims KB, Guelbert N, Cismondi IA, et al. Neuronal ceroid lipofuscinosis type CLN2: a new rationale for the construction of phenotypic subgroups based on a survey of 25 cases in South America. *Gene.* (2013) 516:114–21. doi: 10.1016/j.gene.2012.12.058

52. Gao H, Boustany R-MN, Espinola JA, Cotman SL, Srinidhi L, Antonellis KA, et al. Mutations in a novel CLN6-encoded transmembrane protein cause variant neuronal ceroid lipofuscinosis in man and mouse. *Am J Hum Genet.* (2002) 70:324–35. doi: 10.1086/338190

53. Wheeler RB, Sharp JD, Schultz RA, Joslin JM, Williams RE, Mole SE. The gene mutated in variant late-infantile neuronal ceroid lipofuscinosis (CLN6) and in nclf mutant mice encodes a novel predicted transmembrane protein. *Am J Human Genet.* (2002) 70:537–42. doi: 10.1086/338708

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Cardiac magnetic resonance findings in neuronal ceroid lipofuscinosis: A case report

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Cardiac magnetic resonance imaging (MRI) is an essential tool for the study of hypertrophic cardiomyopathies (HCM) and for differentiating HCM from conditions with increased ventricular wall thickness, such as cardiac storage diseases. Although cardiac MRI is already used for the diagnosis and characterization of some forms of storage diseases involving the myocardium, it has not yet been used to study myocardial involvement in neuronal ceroid lipofuscinosis (NCL). Here, we describe comprehensive cardiac MRI findings in a patient with the CLN3 form of NCL showing basal inferior interventricular septal hypertrophy with maintained indexed LV mass within reference values and low T1-native values. MRI findings support a finding of abnormal storage material within the myocardium in CLN3 disease. We recommend the possible routine use of cardiac MRI for early diagnosis of cardiac involvement in CLN3 disease (also termed juvenile NCL) and to monitor the effects of emerging CLN3 therapies on the myocardium as well.

KEYWORDS

neuronal ceroid lipofuscinosis, batten disease, CLN3, cardiac pathology, cardiac magnetic resonance

Introduction

Cardiac magnetic resonance imaging (MRI) is an essential tool for studying hypertrophic cardiomyopathies (HCM) and to differentiate HCM from conditions presenting with an increase in ventricular wall thickness, such as cardiac deposit diseases (1). Late gadolinium enhancement (LGE) represents the standard for non-invasive imaging of replacement fibrosis (2). Additionally, native T1 mapping without contrast administration improves our diagnostic power also in subjects with contraindications to gadolinium (3). Briefly, high values of native T1 can be obtained in the presence of myocardial fibrosis and edema (4), while low values can be a consequence of the accumulation of iron or fat (4). Cardiac MRI is also used in the diagnosis of Anderson-Fabry disease (AFD) cardiomyopathy, a lysosomal storage disease (LSD) characterized by intracellular accumulation of glycosphingolipids (5). Cardiac involvement in AFD includes left ventricle (LV) hypertrophy, valvular thickening, and

conduction disturbances, followed by heart failure due to myocardial fibrosis induced by glycosphingolipid accumulation (6). Typical LGE pattern suggesting that AFD diagnosis is the late myocardial enhancement localized at the infero-postero-lateral region with the unaffected endocardium. Furthermore, another finding is a low myocardial non-contrast T1 that could be used as a marker to detect myocardial glycosphingolipid storage (7).

Because of these properties, the role of cardiac MRI can be justified for tissue characterization of other forms of storage diseases involving the myocardium. Neuronal ceroid lipofuscinoses (NCL) is a group of autosomal recessive forms of LSD that can present with a combination of epilepsy, psychomotor decline, dementia, and blindness (8). Interestingly, CLN3/juvenile NCL can be complicated by cardiac involvement in the late stages (9). Anecdotal cases reported changes in myocardial function and storage material accumulation in cardiomyocytes of CLN3 patients, among the commonest NCL (10). A study involving 29 CLN3 children reported progressive cardiac involvement with repolarization disturbances at ECG, ventricular hypertrophy, and sinus node dysfunction, and showed an association between inverted T waves and increased risk of death (11). Results from another study involving 42 CLN3 patients evaluated every 6 months by ECG and echocardiography reported hypertrophy and bradycardia as the most common cardiac abnormalities (9). Histopathological analyses on human biopsies showed cytoplasm vacuolization and auto-fluorescent storage material in the cardiomyocytes, especially in the conduction system (12–14) with fibrosis (13), fatty infiltration (13), and calcifications (12). Electron microscopy showed curvilinear bodies, fingerprint patterns, and lipofuscin deposits in cardiomyocytes (15). Here, we describe for the first-time cardiac involvement in a patient with CLN3 disease focusing on features observed on cardiac MRI.

Case description

Patient

A 16-year-old boy with the juvenile phenotype of CLN3 disease underwent a contrast-enhanced cardiac MRI including a native T1 mapping assessment. Informed consent was obtained from the patient's parents. He had an unremarkable prenatal and perinatal history. He could sit unassisted at 6 months, walk unsupported at 18 months, and speak first words at 12 months. Progressive visual impairment began at 5 years with a diagnosis of retinal dystrophy. The first generalized tonic-clonic seizures appeared at 7 years. Epilepsy was partially controlled with valproate (900 mg/die). At that age, brain MRI was reportedly normal. Shortly afterward, progressive psychomotor regression, irregular sleep-wake rhythm, and behavioral disturbances started. ECG and echocardiogram performed annually from the age of 7 were always normal. DNA studies revealed biallelic mutations in *CLN3*, the c.558_559delAG inherited from the father and the c.461-1G>C on the maternal allele (Figure 1). At the latest neurological exam, the patient walked a few steps with support, presenting severe spastic tetraparesis with dystonic postures and cerebellar ataxia. We also recorded blindness, dysphagia, severe cognitive decline, and dysarthria with the production of a few poorly intelligible words. Brain MRI documented mild diffuse and symmetrical hyperintensity in T2-weighted images involving cerebral and cerebellar white matter, thinning of the corpus callosum, and of the optic chiasm, global supra- and sub-tentorial atrophy. EEG detected slow background activity and paroxysmal abnormalities involving mainly the parietal, temporal, and occipital regions bilaterally. At 16 years, ECG showed sinus rhythm with normal atrioventricular conduction, and negative T waves in DIII, V5, V6 leads, and normalized QT interval in the reference range. Two-dimensional transthoracic echocardiography detected mild LV hypertrophy (end-diastolic interventricular septum wall thickness 12 mm) and normal LV ejection fraction (EF 66%, Simpson rule).

Cardiac MRI acquisition and analysis

Cardiac MRI, using a 1.5 Tesla system (GE Healthcare SIGNA Artist) confirmed normal indexed biventricular volumes for age and BSA, and systolic function (EF 69%), with hypertrophy of basal inferior interventricular septum (12 mm) but maintaining indexed LV mass in reference values (74 g/m^2) (Figure 2). Before the administration of the contrast agent, a sequence MOLLI SSFP for native T1 mapping was performed. Native T1 values were calculated by dedicated postprocessing software (Circle Cardiovascular Imaging, Alberta, Canada) and significantly shortened than normal values of the Laboratory were obtained (global T1 of LV was 885 ms, normal value $\geq$ 928 ms) (Figure 3) (16). No areas of LGE (defined as hyperintense areas after administration of gadoteric acid $\geq$ 6 standard deviations than a region of interest in the background) were detected in the myocardium, covering the entire left ventricle from the mitral valve plane to the apex (Figure 4).

Discussion

We presented comprehensive cardiac MRI findings in a patient with CLN3 disease. We found basal inferior interventricular septal hypertrophy with maintained indexed LV mass within reference values and low T1-native values. Our findings in this case of CLN3 disease confirm that some types of NCL are characterized by cardiac involvement with excess storage of ceroid and lipofuscin-like materials in cardiomyocytes (12–14). As in patients with AFD, low myocardial non-contrast

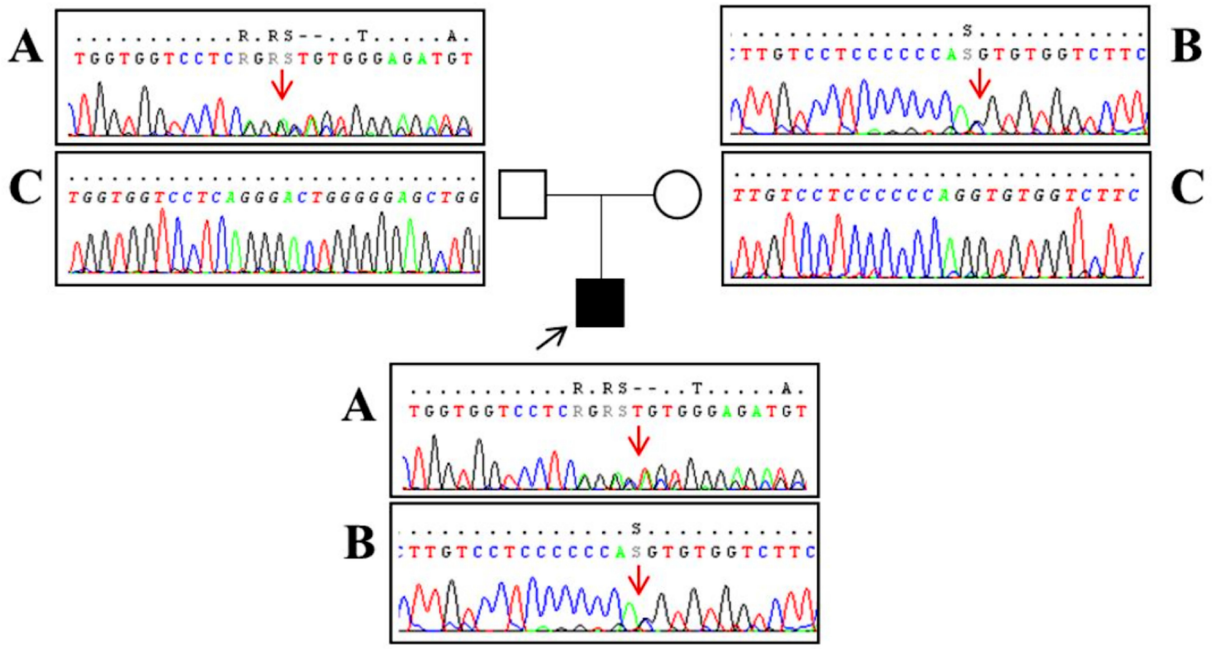

FIGURE 1
Sanger sequencing analysis performed in our patient (black square) revealed a two-base deletion (c.558_559delAG) in exon 8 of the CLN3 gene [**(A)** red arrow] and a splice-site mutation (c.461-1G>C) upstream exon 7 [**(B)** red arrow]. Segregation analysis showed that the father (empty square) carries the c.558_559delAG mutation **(A)** and a wild-type allele **(C)** whereas the mother (empty circle) has the splice-site mutation **(B)** and a wild-type allele **(C)**. Base positions are referred to as the NM_000086 reference sequence (Ensembl genome browser, https://www.ensembl.org/).

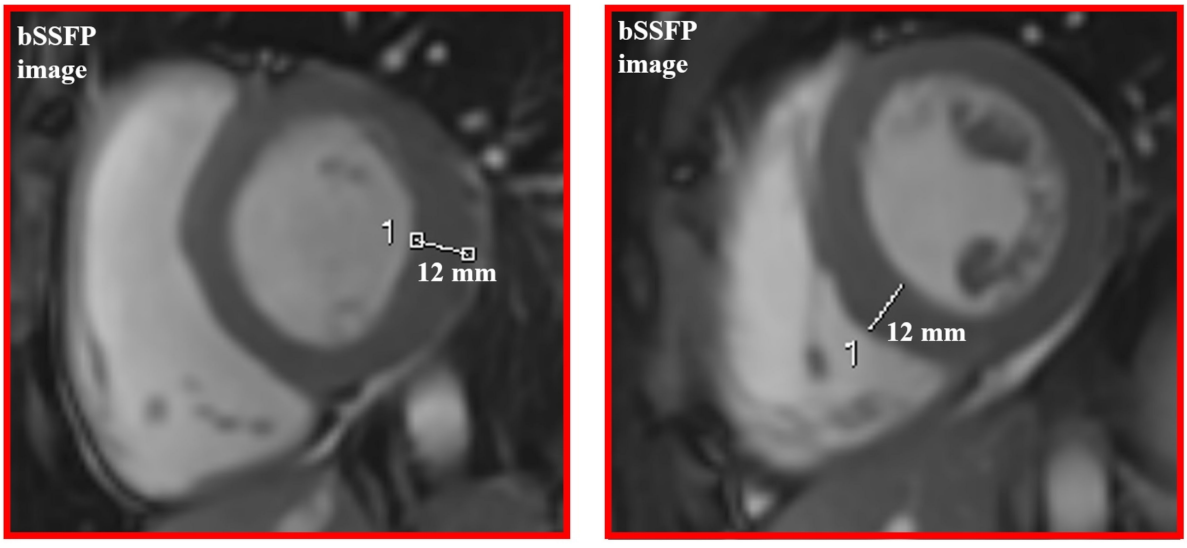

FIGURE 2
Concentric left ventricular hypertrophy of basal and mid segments on cine images.

T1 observed in our patient could be used as a surrogate marker to detect myocardial storage also in CLN3 disease (7). While cardiac function in CLN3 patients has so far been assessed mainly by ECG and echocardiogram, the clinical use of cardiac MRI should be considered as a tool to directly and non-invasively characterize myocardial structure in the early stages

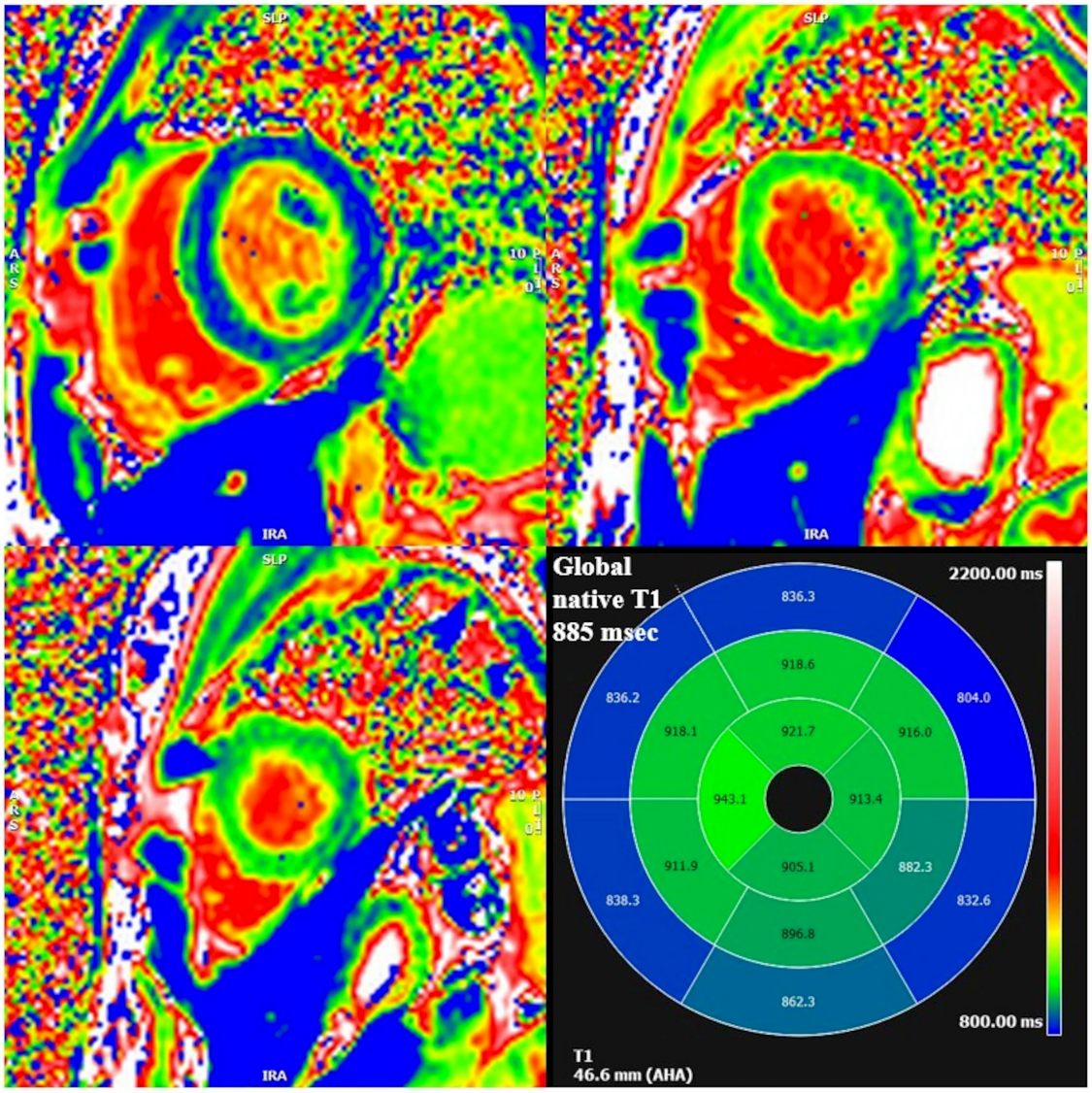

FIGURE 3
Segmental and global low native T1 mapping at MOLLI images. On the bottom right square, AHA segmentation with T1 native values of each segment. Areas of low native T1 are highlighted in blue.

of CLN3 disease, even before life-threatening events, such as advanced conduction disturbances. It is tempting to suggest the routine use of cardiac MRI for the early diagnosis of cardiac involvement in CLN3 disease and to monitor the effects of emerging therapies for CLN3 disease on the myocardium as well. However, it should be noted that a limitation in the routine use of cardiac MRI in CLN3 may be the need for sedation in pediatric patients or patients with advanced dementia.

It should be noted that our patient's genotype does not include the more frequent 1 kb founder deletion, which accounts for approximately 90% of the affected alleles in CLN3 patients (74% homozygous and 22% heterozygous) (17, 18) and leads to the formation of a protein lacking exons 7 and 8, located in the second of the four luminal loops of the protein (19). On the contrary, our patient has an unusual CLN3 genotype with a deletion (c.558_559delAG) in exon 8 and a splice-site mutation (c.461-1G > C) upstream exon 7 of the *CLN3* gene. Both mutations present in our patient have already been described; the 2bp deletion results in a frameshift at the protein level (p.Gly187Aspfs*48) (17), while the second is a mutation in intron 6 that is predicted to result in defective splicing (17). Many compound heterozygous mutations, including the one described here, fall within the same loop involved by 1 kb deletion, suggesting that this domain is critical for protein

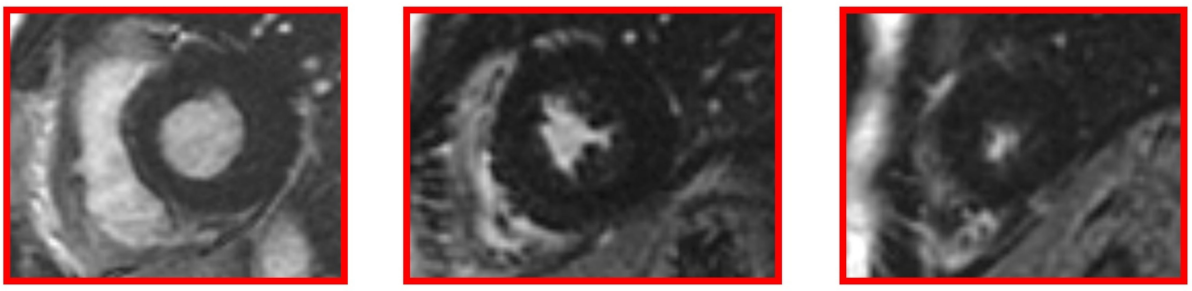

FIGURE 4
Late gadolinium enhancement (LGE) images without hyperintense myocardial areas at the left ventricle.

structure and function (17). Although milder phenotypes with slower progression have been observed, like some missense mutations (19), genotype–phenotype correlation is not well understood, neither regarding the severity of the clinical phenotype (19) nor regarding cardiac involvement. In this regard, one study established that genotype does not predict the severity of behavioral phenotype in CLN3 disease (20). Although there is a need to study cardiac MRI features even in patients with the more frequent 1 kb deletion, it is worth noting that one of the largest studies on cardiac involvement in CLN3 disease showed a high frequency of cardiac ventricular hypertrophy in their 3rd decade of life (11), a finding also confirmed in another cohort of CLN3 patients (9), suggesting that our findings are not attributable to our patient-specific mutation.

Furthermore, our patient obtained subjective benefits from the administration of Trehalose–a sugar being tested in several neurodegenerative diseases, such as NCLs (*ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/results?cond=trehalose&term=&cntry=&state=&city=&dist=; accessed 19 June 2022*) for its neuroprotective actions (21) and its ability to stimulate autophagy (22). In fact, an alteration of the autophagy-lysosome system has been found in many of these conditions. This is also the case with CLN3 disease. Research in various model organisms has highlighted the importance of CLN3 protein function in autophagy, indicating that it influences the expression and activity of lysosomal enzymes and modulates vesicular trafficking and autophagic degradation (23). It is not known whether Trehalose administration may also have a benefit on cardiac function. However, a new MRI study on treated patients is warranted to define any positive effects on the heart as well.

In the present study, cardiac biomarkers were not assessed when cardiac imaging was performed. Troponin and NTproBNP are suggestive of infiltration in subjects with suspected disease and normal left ventricular ejection fraction (LVEF). When cardiac abnormalities are detected by imaging techniques (either ultrasounds or cardiac MRI), their role becomes relevant during follow-up, to assess the progression of the disease and the possible effect of treatment.

Being a case report, the results are limited to a single case with an unusual genotype, thus firm conclusions cannot be drawn. Presentation of cardiac MRI results on a single patient and the absence of a baseline cardiac MRI examination are the main weak points, though the study brings original information worth exploring further. The strength is that, to our best knowledge, this is the first study of NCL-associated heart disease by cardiac MRI.

Data availability statement

The datasets presented in this article are not readily available because of ethical and privacy restrictions. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the CEPR-Tuscany Region Pediatric Ethics Committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the participants' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

GT and FS conceived the experiment. GT, MM, and AB performed cardiac MR analysis. SD, MM, IR, EC, and AT collected clinical, imaging, and genetic data. AT performed the genetic analysis. GT wrote the first draft of the manuscript. All

authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Authors/Task Force members, Elliott PM, Anastasakis A, Borger MA, Borggrefe M, Cecchi F, et al. 2014 ESC guidelines on diagnosis and management of hypertrophic cardiomyopathy: the task force for the diagnosis and management of hypertrophic cardiomyopathy of the european society of cardiology (ESC). *Eur Heart J.* (2014) 35:2733–79. doi: 10.1093/eurheartj/ehu284

2. Kim RJ, Wu E, Rafael A, Chen EL, Parker MA, Simonetti O, et al. The use of contrast-enhanced magnetic resonance imaging to identify reversible myocardial dysfunction. *N Engl J Med.* (2000) 343:1445–53. doi: 10.1056/NEJM200011163432003

3. Moon JC, Messroghli DR, Kellman P, Piechnik SK, Robson MD, Ugander M, et al. Myocardial T1 mapping and extracellular volume quantification: a Society for Cardiovascular Magnetic Resonance (SCMR) and CMR Working Group of the European Society of Cardiology consensus statement. *J Cardiovasc Magn Reson.* (2013) 15:92. doi: 10.1186/1532-429X-15-92

4. Germain P, El Ghannudi S, Jeung MY, Ohlmann P, Epailly E, Roy C, et al. Native T1 mapping of the heart-a pictorial review. *Clin Med Insights Cardiol.* (2014) 8:1–11. doi: 10.4137/CMC.S19005

5. Karur GR, Robison S, Iwanochko RM, Morel CF, Crean AM, Thavendiranathan P, et al. Use of Myocardial T1 Mapping at 30 T to differentiate Anderson-Fabry disease from hypertrophic cardiomyopathy. *Radiology.* (2018) 288:398–406. doi: 10.1148/radiol.2018172613

6. Smid BE, van der Tol L, Cecchi F, Elliott PM, Hughes DA, Linthorst GE, et al. Uncertain diagnosis of Fabry disease: consensus recommendation on diagnosis in adults with left ventricular hypertrophy and genetic variants of unknown significance. *Int J Cardiol.* (2014) 177:400–8. doi: 10.1016/j.ijcard.2014.09.001

7. Sado DM, White SK, Piechnik SK, Banypersad SM, Treibel T, Captur G, et al. Identification and assessment of Anderson-Fabry disease by cardiovascular magnetic resonance noncontrast myocardial T1 mapping. *Circ Cardiovasc Imaging.* (2013) 6:392–8. doi: 10.1161/CIRCIMAGING.112.000070

8. Mole SE, Cotman SL. Genetics of the neuronal ceroid lipofuscinoses (Batten disease). *Biochim Biophys Acta.* (2015) 1852:2237–41. doi: 10.1016/j.bbadis.2015.05.011

9. Rietdorf K, Coode EE, Schulz A, Wibbeler E, Bootman MD, Ostergaard JR, et al. Cardiac pathology in neuronal ceroid lipofuscinoses (NCL): More than a mere co-morbidity. *Biochim Biophys Acta Mol Basis Dis.* (2020) 1866:165643. doi: 10.1016/j.bbadis.2019.165643

10. Sørensen JB, Parnas J. A clinical study of 44 patients with juvenile amaurotic family idiocy. *Acta Psychiatr Scand.* (1979) 59:449–61. doi: 10.1111/j.1600-0447.1979.tb00244.x

11. Ostergaard JR, Rasmussen TB, Mølgaard H. Cardiac involvement in juvenile neuronal ceroid lipofuscinosis (Batten disease). *Neurology.* (2011) 76:1245–51. doi: 10.1212/WNL.0b013e31821435bd

12. Reske-Nielsen E, Baandrup U, Bjerregaard P, Bruun I. Cardiac involvement in juvenile amaurotic idiocy–a specific heart muscle disorder. Histological findings in 13 autopsied patients. *Acta Pathol Microbiol Scand A.* (1981) 89:357–65. doi: 10.1111/j.1699-0463.1981.tb00233.x

13. Hofman IL, van der Wal AC, Dingemans KP, Becker AE. Cardiac pathology in neuronal ceroid lipofuscinoses–a clinicopathologic correlation in three patients. *Eur J Paediatr Neurol.* (2001) 5:213–7. doi: 10.1053/ejpn.2000.0465

14. Michielsen P, Martin JJ, Vanagt E, Vrints C, Gillebert T, Snoeck J. Cardiac involvement in juvenile ceroid lipofuscinosis of the Spielmeyer-Vogt-Sjögren type: prospective noninvasive findings in two siblings. *Eur Neurol.* (1984) 23:166–72. doi: 10.1159/000115699

15. Gilbert-Barness E. Review: Metabolic cardiomyopathy and conduction system defects in children. *Ann Clin Lab Sci.* (2004) 34:15–34.

16. Meloni A, Martini N, Positano V, D'Angelo G, Barison A, Todiere G, et al. Myocardial T1 Values at 1.5 T: Normal Values for General Electric Scanners and Sex-Related Differences. *J Magn Reson Imaging.* (2021) 54:1486–500. doi: 10.1002/jmri.27639

17. Mirza M, Vainshtein A, DiRonza A, Chandrachud U, Haslett LJ, Palmieri M, et al. The CLN3 gene and protein: what we know. *Mol Genet Genomic Med.* (2019) 7:e859. doi: 10.1002/mgg3.859

18. Gardner E, Mole SE. The genetic basis of phenotypic heterogeneity in the neuronal ceroid lipofuscinoses. *Front Neurol.* (2021) 12:754045. doi: 10.3389/fneur.2021.754045

19. Kousi M, Lehesjoki AE, Mole SE. Update of the mutation spectrum and clinical correlations of over 360 mutations in eight genes that underlie the neuronal ceroid lipofuscinoses. *Hum Mutat.* (2012) 33:42–63. doi: 10.1002/humu.21624

20. Adams HR, Beck CA, Levy E, Jordan R, Kwon JM, Marshall FJ, et al. Genotype does not predict severity of behavioural phenotype in juvenile neuronal ceroid lipofuscinosis (Batten disease). *Dev Med Child Neurol.* (2010) 52:637–43. doi: 10.1111/j.1469-8749.2010.03628.x

21. Lee HJ, Yoon YS, Lee SJ. Mechanism of neuroprotection by trehalose: controversy surrounding autophagy induction. (2018). 9:712. doi: 10.1038/s41419-018-0749-9

22. Rusmini P, Cortese K, Crippa V, Cristofani R, Cicardi ME, Ferrari V, et al. Trehalose induces autophagy via lysosomal-mediated TFEB activation in models of motoneuron degeneration. *Autophagy.* (2019) 15:631–51. doi: 10.1080/15548627.2018.1535292

23. Kim WD, Wilson-Smillie M, Thanabalasingam A, Lefrancois S, Cotman SL, Huber RJ. Autophagy in the Neuronal Ceroid Lipofuscinoses (Batten Disease). *Front Cell Dev Biol.* (2022) 10:812728. doi: 10.3389/fcell.2022.812728

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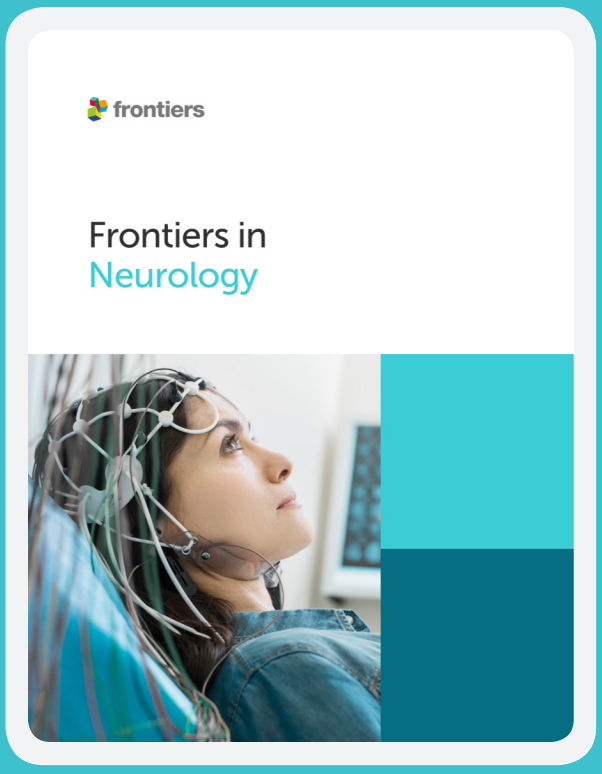

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