BBA - Molecular Basis of Disease 1866 (2020) 165681

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Review

Future perspectives: What lies ahead for Neuronal Ceroid Lipofuscinosis research?

Jonathan D. Cooper[a,*], Sara E. Mole[b]

[a] Pediatric Storage Disorders Laboratory, Department of Pediatrics, Division of Genetics and Genomics, Washington University in St. Louis, School of Medicine, St Louis, MO 63110, USA

[b] UCL MRC Laboratory for Molecular Cell Biology and UCL Great Ormond Street Institute of Child Health, University College London, London WC1E 6BT, United Kingdom

ARTICLE INFO

Keywords:
Batten disease
Neuronal Ceroid Lipofuscinoses
International congress
Future perspectives

ABSTRACT

Progress is being made in all aspects of Neuronal Ceroid Lipofuscinosis (NCL) research, resulting in many recent advances. These advances encompass several areas that were previously thought intractable, ranging from basic science, through to a better understanding of the clinical presentation of different forms of NCL, therapeutic development, and new clinical trials that are underway. Increasing numbers of original NCL research papers continue to be published, and this new sense of momentum is greatly encouraging for the field. Here, we make some predictions as to what we can anticipate in the next few years.

1. Introduction

The increasing pace of progress in all aspects of Neuronal Ceroid Lipofuscinosis (NCL) research is highlighted by the breadth and depth of review articles [1–8] in this Special Issue that provide a helpful snapshot of the field between the previous international meeting, NCL 2018, and the next, NCL 2020. Here, we make some predictions as to what we can anticipate in the next few years.

2. Advances in NCL cell biology

Despite the identification of many of the disease-causing gene mutations [9,10], progress towards developing effective therapies for the NCLs has long been hampered by a relatively poor understanding of the underlying disease mechanisms [6,11-12]. However, the cell biology of the deficient proteins continues to emerge [1,12], with the recent identification of the CLN8 protein as an endoplasmic reticulum cargo receptor that regulates lysosomal biogenesis [13]. It is anticipated that further insights will soon emerge for other forms of NCL, including the long-elusive function of CLN3 and how this is affected by mutation. Such information will not only shed much needed light on disease mechanisms, but will reveal the true extent to which the different forms of NCL are similar or distinct from one another. Such advances in understanding pathogenesis will aid the development of mechanistic-based treatments, and increase the means to intervene therapeutically [9,14]. Nevertheless, many basic questions remain unanswered. For example, while the biological actions of the enzymes deficient in CLN1 and CLN2 disease have long been known [15], their normal substrates remain unidentified. Even the precise intracellular location of action for several NCL proteins remains unclear [15], with the PPT1 enzyme showing a pH optimum that does not match its predicted lysosomal location [16]. Answering, such fundamental questions about the normal function of these proteins, or even identifying where inside the cell their interactions primarily occur, both remain significant challenges.

3. The cellular-specificity of disease

Until recently, most of the attention of NCL researchers has understandably been upon the brain, and more specifically upon the neurons within it. While it has long been apparent that specific types of neurons are affected to different extents [6,11], the reasons underlying this selective vulnerability in these disorders are still not known. Indeed, while many of these vulnerable neuron populations exist in interconnected pathways, whether disease actually spreads anterogradely along these pathways or if this is just a co-incidence is far from clear. Similarly, new insights into the contributory role of glia in contributing to or causing neuron loss in the NCLs have been revealed [17,18], and hint that different forms of NCL are fundamentally distinct from one another in how individual cell types are affected. It will be important to re-evaluate such issues of cell autonomy *in vivo*, rather than depending solely on tissue culture data. Such findings may have significant implications for the efficacy of therapies as certain treatments such as

* Corresponding author.
E-mail address: cooperjd@wustl.edu (J.D. Cooper).

https://doi.org/10.1016/j.bbadis.2020.165681
Received 11 December 2019; Received in revised form 2 January 2020; Accepted 6 January 2020
Available online 08 January 2020
0925-4439/

gene AAV9-mediated gene therapy show strong neuronal tropism [19] which may leave affected glial cells untreated. Nevertheless, such observations raise further questions about how similar these diseases really are, and why they were originally grouped together. Undoubtedly, they broadly share certain clinical and pathological similarities with each other and also with other diseases. However, the more is learnt about each form, the more differences emerge. What seems likely is that these disorders converge upon a similar pathological endpoint involving pronounced neuron loss and autofluorescent storage material accumulation, but are actually distinct disorders with separate underlying molecular causes. In this respect, understanding the consequences of these mutations upon the transcriptome and proteome is likely to be particularly informative.

4. Thinking outside the brain

An important additional consideration is that although the effect of disease-causing mutations is devastating upon the brain, these effects clearly extend to other components of the CNS [20], including the peripheral and autonomic nervous system and to other somatic tissues [21–23]. Such underappreciated effects of disease may underlie some of the most debilitating effects of disease, and treating these successfully may significantly improve the quality of life for affected children and their families. Some of these newly identified effects of disease only became apparent after successfully treating the CNS in pre-clinical studies that prolonged life [20], resulting in new disease phenotypes emerging. Whether these unforeseen effects of disease are confined to the populations of neurons or nerve fibers that are present in such tissues, or extends to other somatic cell types themselves is yet to be properly considered. However, it is likely that such pathological and functional consequences will contribute to disease outcome, and it is clear that these underappreciated effects of disease will need to be treated. The spinal cord appears to be affected in every form of NCL examined so far [6,22], as is the peripheral nervous system, and it remains to be seen whether these events are related to one another. Another emerging theme is the involvement of cardiac dysfunction in multiple forms of NCL [8], most prominently in CLN3 disease [23]. It is anticipated that the extent of such unexpected 'non-brain' effects of disease will continue to be emerge in the years to come, and such findings have significant implications for the targeting of treatments to where they can be most effective. As such understanding whether these events occur secondary to disease in the CNS, or are intrinsic to somatic tissues, will be key for maximizing therapeutic efficacy.

5. Moving beyond mouse models

The vast majority of pre-clinical studies, whether assessing pathogenesis or therapeutic efficacy, have utilized a series of NCL mouse models [4,24-25]. As a well-understood model species, NCL mice have proved valuable for defining the effects of disease and obtaining *proof-of-principle* evidence of efficacy. However, it is now becoming more apparent that mice have severe limitations in modelling the full extent of human disease, and there are potential risks inherent in progressing to clinical trials based solely on data from mice. In this respect, larger animal species such as sheep, dogs and pigs have a crucial role to play in refining the dosing and delivery routes for therapeutic strategies before being considered for clinical trials [4,25,26]. The advent of new genome editing methods such as CRISPR/Cas 9 has enabled the efficient generation of animal models with a disease-causing mutation of choice. Recently, such methods have been used to generate both CLN3 pig [27] and CLN1 sheep [28] models of disease, which more closely recapitulate human disease phenotypes that are not present in mouse models. Characterizing these new larger animal models, including obtaining detailed behavioral, radiological and pathological landmarks of disease progression [26] will be needed, as has been done for the mouse models of NCLs. Such studies will also shed light on pathomechanisms that may not be recapitulated in mouse models and extend the basis for assessing the efficacy of therapeutic approaches. Indeed, such larger animal models are expected to be ideally suited for scaling-up the delivery and dosing of therapeutic strategies and represent a key staging post between mouse studies and designing effective clinical trials.

6. A new translational landscape

Perhaps most significantly, the translational landscape of these disorders has changed dramatically in recent years with the first ever FDA-approved therapy [29] (*Brineura*, enzyme replacement for CLN2 disease), which is significantly improving quality of life and delaying disease progression for persons affected by this disease [30]. This translational momentum has accelerated with Phase I/IIa clinical trials of gene therapy currently in progress for two other forms of NCL (CLN3 disease: NCT03770572 [31]; CLN6 disease NCT02725580 [32]). It was always known that gene therapy for transmembrane protein-deficient NCLs would be challenging, as 'cross correction' is not possible as it is for enzyme deficient forms of NCL. Nevertheless, the advent of newer generations of vectors has facilitated the more efficient and widespread transduction of tissues within the CNS. An important breakthrough has been the application of such gene therapy methods to treating retinal pathology [33], and the recognition that multiple key cell populations may need to be targeted to prevent the death of others. This may require new vector serotypes and the identification of cell-type specific promotors for driving therapeutic gene expression in these clinically relevant tissues. It is also possible that in the future gene therapy will not be confined to expressing a functional copy of deficient genes, but may be used to deliver new gene editing tools to correct disease-causing mutations. Nevertheless, the potential for off target effects still exists, and future studies will need to take these into account.

7. Adopting a long-term perspective

Another major challenge for gene therapy approaches is the presence of naturally occurring antibodies against adeno-associated viruses (AAVs) that present in a variable proportion of the patient population [34]. It also remains to be seen what the longer-term consequences of gene therapy will be. Given that gene therapy is generally considered a 'one off' single time treatment, the issue of whether transgene expression is sustained in the long term will be important to address, with re-administration of vectors unlikely to be feasible with current technology. Similar longer-term perspectives apply to enzyme replacement approaches. While *Brineura* appears effective in halting disease progression in CLN2 disease children [30], and relatively few adverse events have been reported so far, it is unclear how these individuals will continue to respond to ERT over ten or more years. However, considering the previously bleak outlook for these children, the fact that the field is now in a position to ask such questions regarding longer-term therapeutic efficacy is remarkable in itself.

8. The importance of working together

With significant progress continuing to be made for a variety of other pre-clinical interventions in other forms of NCL [14,35], further clinical trials are anticipated in the near future, including those that extend the detailed natural history of these disorders. In this respect, patient registries and detailed rating schemes will continue to prove essential, and the active participation of affected families in these studies will continue to be key to their success. Indeed, many of the advances in NCL research have been achieved through significant cross-stakeholder collaboration between patient advocacy groups, academic researchers, clinicians, and industry scientists. Such collaboration is essential to this progress.

9. International Congresses and focused workshops

Many of these interactions were initiated and strengthened at the biennial International Congresses on Neuronal Ceroid Lipofuscinosis (Batten disease), with the most recent being NCL2018 held in London, UK. The next meeting, NCL 2020, will be held at Washington University in St Louis, MO, USA, from October 7–11, 2020. These international Congresses facilitate communication of the latest advances in basic and clinical science, and the identification of remaining gaps in knowledge and how these may be overcome. However, with the pace of progress in NCL research increasing in both pre-clinical and clinical studies, the introduction of focused patient-group initiated *Translational NCL Research* meetings in the intervening years between International congresses has proved invaluable for driving the field forwards.

10. Final comments

At present we stand at the beginning of a new age of NCL research, and as its focus becomes increasingly translational, the challenges we face will continue to evolve accordingly. It is the responsibility of all involved to meet these challenges in order to realize the full potential of this promising progress.

Declaration of competing interest

JDC has been in receipt of research support from BioMarin Pharmaceutical Inc., Abeona Therapeutics Inc., Regenexbio Inc., CereSpir Inc., SEM receives financial support from BioMarin Pharmaceutical Inc. to maintain the NCL Mutation Database and acts as an advisor to BioMarin Pharmaceutical Inc.

Acknowledgements

We would like to thank Drs. Alison Barnwell and Hemanth Ramesh Nelvagal for their constructive comments on the manuscript.

References

[1] R.A. Kline, T.M. Wishart, K. Mills, W.E. Heywood, Applying modern omic technologies to the neuronal ceroid lipofuscinoses, Biochim Biophys Acta Mol Basis Dis. (2019) 165498, , https://doi.org/10.1016/j.bbadis.2019.06.012.

[2] B. Elmerskog, A.G. Tøssebro, R. Atkinson, S. Rokne, B. Cole, A. Ockelford, H.R. Adams, Overview of advances in educational and social supports for young persons with NCL disorders, Biochim. Biophys. Acta Mol. Basis Dis. 165480 (2019), https://doi.org/10.1016/j.bbadis.2019.05.016.

[3] C.J. Minnis, C.D. Thornton, L.M. FitzPatrick, T.R. McKay, Cellular models of Batten disease, Biochim. Biophys. Acta Mol. Basis Dis. 165559 (2019), https://doi.org/10.1016/j.bbadis.2019.165559.

[4] R.J. Huber, S.M. Hughes, W. Liu, A. Morgan, R.I. Tuxworth, C. Russell, The contribution of multicellular model organisms to neuronal ceroid lipofuscinosis research, Biochim. Biophys. Acta Mol. Basis Dis. 165614 (2019), https://doi.org/10.1016/j.bbadis.2019.165614.

[5] V. Kauss, M. Dambrova, D.L. Medina, Pharmacological approaches to tackle NCLs, Biochim. Biophys. Acta Mol. Basis Dis. 165553 (2019), https://doi.org/10.1016/j.bbadis.2019.165553.

[6] H.R. Nelvagal, J. Lange, K. Takahashi, M.A. Tarczyluk-Wells, J.D. Cooper, Pathomechanisms in the neuronal ceroid lipofuscinoses, Biochim. Biophys. Acta Mol. Basis Dis. 165570 (2019), https://doi.org/10.1016/j.bbadis.2019.165570.

[7] E.S. Butz, U. Chandrachud, S.E. Mole, S.L. Cotman, Moving towards a new era of genomics in the neuronal ceroid lipofuscinoses, Biochim Biophys Acta Mol Basis Dis. 165571 (2019), https://doi.org/10.1016/j.bbadis.2019.165571.

[8] A. Schulz, K. Rietdorf, E.E. Coode, E. Wibbeler, M.D. Bootman, J.R. Ostergaard, Cardiac pathology in neuronal ceroid lipofuscinoses (NCL): More than a mere co-morbidity, Biochim Biophys Acta Mol Basis Dis. 165643 (2019), https://doi.org/10.1016/j.bbadis.2019.165643.

[9] S.E. Mole, G. Anderson, H.A. Band, S.F. Berkovic, J.D. Cooper, S.M. Kleine Holthaus, T.R. McKay, D.L. Medina, A.A. Rahim, A. Schulz, A.J. Smith, Clinical challenges and future therapeutic approaches for neuronal ceroid lipofuscinosis, Lancet Neurol. 18 (2019) 107–116.

[10] S.E. Mole, S.L. Cotman, Genetics of the neuronal ceroid lipofuscinoses (Batten disease), Biochim. Biophys. Acta 1852 (2015) 2237–2241.

[11] J.D. Cooper, M.A. Tarczyluk, H.R. Nelvagal, Towards a new understanding of NCL pathogenesis, Biochim. Biophys. Acta 1852 (2015) 2256–2261.

[12] A.B. Mukherjee, A.P. Appu, T. Sadhukhan, S. Casey, A. Mondal, Z. Zhang, M.B. Bagh, Emerging new roles of the lysosome and neuronal ceroid lipofuscinoses, Mol. Neurodegener. 14 (2019) 4.

[13] A. di Ronza, L. Bajaj, J. Sharma, D. Sanagasetti, P. Lotfi, C.J. Adamski, J. Collette, M. Palmieri, A. Amawi, L. Popp, K.T. Chang, M.C. Meschini, H.E. Leung, L. Segatori, A. Simonati, R.N. Sifers, F.M. Santorelli, M. Sardiello, CLN8 is an endoplasmic reticulum cargo receptor that regulates lysosome biogenesis, Nat. Cell Biol. 20 (2018) 1370–1377.

[14] T.B. Johnson, J.T. Cain, K.A. White, D. Ramirez-Montealegre, D.A. Pearce, J.M. Weimer, Therapeutic landscape for Batten disease: current treatments and future prospects, Nat. Rev. Neurol. 15 (2019) 161–178, https://doi.org/10.1038/s41582-019-0138-8.

[15] J. Cárcel-Trullols, A.D. Kovács, D.A. Pearce, Cell biology of the NCL proteins: What they do and don't do, Biochim Biophys Acta 1852 (2015) 2242–2255, https://doi.org/10.1016/j.bbadis.2015.04.027.

[16] A.K. Das, J.J. Bellizzi, S. Tandel, E. Biehl, J. Clardy, S.L. Hofmann, Structural basis for the insensitivity of a serine enzyme (palmitoyl-protein thioesterase) to phenylmethylsulfonyl fluoride, J. Biol. Chem. 275 (2000) 23847–23851.

[17] L. Parviainen, S. Dihanich, G.W. Anderson, A.M. Wong, H.R. Brooks, R. Abeti, P. Rezaie, G. Lalli, S. Pope, S.J. Heales, H.M. Mitchison, B.P. Williams, J.D. Cooper, Glial cells are functionally impaired in juvenile neuronal ceroid lipofuscinosis and detrimental to neurons, Acta Neuropathol Commun. 5 (2017) 74.

[18] J. Lange, L.J. Haslett, E. Lloyd-Evans, J.M. Pocock, M.S. Sands, B.P. Williams, J.D. Cooper, Compromised astrocyte function and survival negatively impact neurons in infantile neuronal ceroid lipofuscinosis, Acta Neuropathol Commun. 6 (2018) 74.

[19] P.A. Lawlor, R.J. Bland, A. Mouravlev, D. Young, During, efficient gene delivery and selective transduction of glial cells in the mammalian brain by AAV serotypes isolated from nonhuman primates, Mol. Ther. 17 (2009) 1692–1702, https://doi.org/10.1038/mt.2009.170.

[20] C. Shyng, H.R. Nelvagal, J.T. Dearborn, J. Tyynelä, R.E. Schmidt, M.S. Sands, J.D. Cooper, Synergistic effects of treating the spinal cord and brain in CLN1 disease, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) E5920–E5929.

[21] N. Galvin, C. Vogler, B. Levy, A. Kovacs, M. Griffey, M.S. Sands, A murine model of infantile neuronal ceroid lipofuscinosis-ultrastructural evaluation of storage in the central nervous system and viscera, Pediatr. Dev. Pathol. 11 (2008) 185–192.

[22] J.R. Ostergaard, Paroxysmal sympathetic hyperactivity in juvenile neuronal ceroid lipofuscinosis (Batten disease), Auton. Neurosci. 214 (2018) 15–18, https://doi.org/10.1016/j.autneu.2018.07.003.

[23] J.R. Ostergaard, T.B. Rasmussen, H. Mølgaard, Cardiac involvement in juvenile neuronal ceroid lipofuscinosis (Batten disease), Neurology. 76 (2011) 1245–1251, https://doi.org/10.1212/WNL.0b013e31821435bd.

[24] J.J. Shacka, Mouse models of neuronal ceroid lipofuscinoses: useful pre-clinical tools to delineate disease pathophysiology and validate therapeutics, Brain Res. Bull. 88 (2012) 43–57, https://doi.org/10.1016/j.brainresbull.2012.03.003.

[25] M. Bond, S.M. Holthaus, I. Tammen, G. Tear, C. Russell, Use of model organisms for the study of neuronal ceroid lipofuscinosis, Biochim. Biophys. Acta 1832 (2013) 1842–1865, https://doi.org/10.1016/j.bbadis.2013.01.009.

[26] N.L. Mitchell, K.N. Russell, M.P. Wellby, H.E. Wicky, L. Schoderboeck, G.K. Barrell, T.R. Melzer, S.J. Gray, S.M. Hughes, D.N. Palmer, Longitudinal in vivo monitoring of the CNS demonstrates the efficacy of gene therapy in a sheep model of CLN5 batten disease, Mol. Ther. 26 (2018) 2366–2378.

[27] T.B. Johnson, D.A. Sturdevant, K.A. White, A.V. Drack, S. Bhattarai, C. Rogers, J.D. Cooper, D.A. Pearce, J.M. Weimer, Characterization of a novel porcine model of CLN3-Batten disease, Mol. Genet. Metab. 126 (2019) S81, https://doi.org/10.1016/J.YMGME.2018.12.198.

[28] S.L. Eaton, C. Proudfoot, S.G. Lillico, P. Skehel, R.A. Kline, K. Hamer, N.M. Rzechorzek, E. Clutton, R. Gregson, T. King, C.A. O'Neill, J.D. Cooper, G. Thompson, C.B. Whitelaw, T.M. Wishart, CRISPR/Cas9 mediated generation of an ovine model for infantile neuronal ceroid lipofuscinosis (CLN1 disease), Sci. Rep. 9 (2019) 9891.

[29] A. Markham, Cerliponase alfa: first global approval, Drugs. 77 (11) (2017) 1247–1249.

[30] A. Schulz, T. Ajayi, N. Specchio, E. de Los Reyes, P. Gissen, D. Ballon, J.P. Dyke, H. Cahan, P. Slasor, D. Jacoby, A. Kohlschütter, CLN2 Study Group, Study of intraventricular cerliponase alfa for CLN2 disease, N Engl J Med. 378 (2018) 1898–1907.

[31] NCT03770572: Gene Transfer Study of AAV9-CLN3 for Treatment NCL Type 3, Accessed at: https://clinicaltrials.gov/ct2/show/NCT03770572?cond=CLN3&rank=1.

[32] NCT02725580: Batten CLN6 Gene Therapy, Accessed at: https://clinicaltrials.gov/ct2/show/NCT02725580?cond=CLN6&rank=1.

[33] S.M. Kleine Holthaus, J. Ribeiro, L. Abelleira-Hervas, R.A. Pearson, Y. Duran, A. Georgiadis, R.D. Sampson, M. Rizzi, J. Hoke, R. Maswood, S. Azam, U.F.O. Luhmann, A.J. Smith, S.E. Mole, R.R. Ali, Prevention of photoreceptor cell loss in a $Cln6^{nclf}$ mouse model of batten disease requires CLN6 gene transfer to bipolar cells, Mol. Ther. 26 (2018) 1343–1353, https://doi.org/10.1016/j.ymthe.2018.02.027.

[34] K.I. Berns, N. Muzyczka, AAV: an overview of unanswered questions, Hum. Gene Ther. 28 (2017) 308–313, https://doi.org/10.1089/hum.2017.048.

[35] A. Kohlschütter, A. Schulz, U. Bartsch, S. Storch, Current and emerging treatment strategies for neuronal ceroid lipofuscinoses, CNS Drugs. 33 (2019) 315–325.