

# **Future perspectives: what lies ahead for Neuronal Ceroid Lipofuscinosis Research?**

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## **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<sup>1-8</sup> 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<sup>9,10</sup>, progress towards developing effective therapies for the NCLs has long been hampered by a relatively poor understanding of the underlying disease mechanisms<sup>6,11-12</sup>. However, the cell biology of the deficient proteins continues to emerge<sup>1,12</sup>, with the recent identification of the CLN8 protein as an endoplasmic reticulum cargo receptor that regulates lysosomal biogenesis<sup>13</sup>. 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<sup>9,14</sup>. 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<sup>15</sup>, their normal substrates remain unidentified. Even the precise intracellular location of action for several NCL proteins remains unclear<sup>15</sup>, with

the PPT1 enzyme showing a pH optimum that does not match its predicted lysosomal location<sup>16</sup>. 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<sup>6,11</sup>, 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<sup>17,18</sup>, 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 gene AAV9-mediated gene therapy show strong neuronal tropism<sup>19</sup> 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<sup>20</sup>, including the peripheral and autonomic nervous system and to other somatic tissues<sup>21-23</sup>. 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<sup>20</sup>, 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<sup>6,22</sup>, 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<sup>8</sup>, most prominently in CLN3 disease<sup>23</sup>. 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<sup>4,24-25</sup>. 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<sup>4,25,26</sup>. 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<sup>27</sup> and CLN1 sheep<sup>28</sup> 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<sup>26</sup> 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<sup>29</sup> (*Brineura*, enzyme replacement for CLN2 disease), which is significantly improving quality of life and delaying disease progression for persons affected by this disease<sup>30</sup>. 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<sup>31</sup>; CLN6 disease NCT02725580<sup>32</sup>). 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<sup>33</sup>, 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 promoters 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<sup>34</sup>. 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<sup>30</sup>, 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<sup>14,35</sup>, 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.

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