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Review

Towards a new understanding of NCL pathogenesis☆

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ABSTRACT

The Neuronal Ceroid Lipofuscinoses (NCLs, Batten disease) are a group of inherited neurodegenerative disorders that have been traditionally grouped together on the basis of certain shared clinical and pathological features. However, as the number of genes that appear to cause new forms of NCL continues to grow, it is timely to reassess our understanding of the pathogenesis of these disorders and what groups them together. The various NCL subtypes do indeed share features of a build-up of autofluorescent storage material, progressive neuron loss and activation of the innate immune system. The characterisation of animal models has highlighted the selective nature of neuron loss and its intimate relationship with glial activation, rather than the generalised build-up of storage material. More recent data provide evidence for the pathway-dependent nature of pathology, the contribution of glial dysfunction, and the involvement of new brain regions previously thought to be unaffected, and it is becoming apparent that pathology extends beyond the brain. These data have important implications, not just for therapy, but also for our understanding of these disorders. However, looking beneath these broadly similar pathological themes evidence emerges for marked differences in the nature and extent of these events in different forms of NCL. Indeed, given the widely different nature of the mutated gene products it is perhaps more surprising that these disorders resemble each other as much as they do. Such data raise the question whether we should rethink the collective grouping of these gene deficiencies together, or whether it would be better to consider them as separate entities. This article is part of a Special Issue entitled: Current Research on the Neuronal Ceroid Lipofuscinoses (Batten Disease).

1. Introduction

As detailed elsewhere in this special issue [62], considerable progress has been made in identifying the genes that are mutated in the series of inherited neurodegenerative disorders collectively called the Neuronal Ceroid Lipofuscinoses (NCLs, or Batten Disease). This grouping together is largely on the apparent similarities in their clinical presentation [81] and brain pathology [1,77,98], despite their widely disparate ages of onset and rates of disease progression. Perhaps the characteristic defining pathological feature of these disorders is the intralysosomal accumulation of a complex mixture of proteins, lipids and metals, which have characteristic autofluorescent properties [70,71]. Previously, the ultrastructural appearance of this stored material was used diagnostically, in combination with the clinical presentation. However, more recently the availability of enzymatic assays and the identification of many new disease-causing mutations have enabled more rapid and reliable diagnoses of the different disease subtypes [106]. Indeed, in recent years, the availability and affordability of modern genomic methodology have seen the identification of a plethora of new disease forms [2,8,66,85,87,88,92], which display a similar clinical presentation and autofluorescent storage material accumulation. The hypothesised number of disease subtypes has expanded rapidly and currently stands at fourteen different forms of NCL [62,85].

Recently, a new classification of these disorders has emerged, which combines the mutated gene and age of onset [105]. Nevertheless, despite knowing the genetic basis of these disorders [62,102], progress towards understanding how these mutations exert their devastating effects has been frustratingly slow. With relatively little known about the normal function of most of the gene products [12,45], it has been difficult to determine how this may be disrupted in each disease subtype, and many fundamental questions remain unanswered. For example, which of the many phenotypes that have been reported represent primary consequences of mutation, and which are more secondary or further downstream parts of a disease cascade that progressively becomes ever more wide reaching? These are key issues that have direct relevance for devising therapeutic strategies.

The existence of an uptake mechanism for soluble enzyme deficient forms of NCL [53,89,99], means that a detailed knowledge of the normal function of these gene products and the consequences of their disruption may be less crucial for moving towards an effective therapy. While this information would still be valuable to have, as reviewed elsewhere in this special issue [65], therapeutic efforts can instead concentrate on the considerable technical challenges of how to deliver these

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missing proteins, whether by direct enzyme replacement [e.g. [13,37,54, 58,59,101], gene therapy [31,32,56,74,90,91], or via neural stem cell transplantation [93]].

In contrast, distinguishing which are the key events that happen following gene mutation is of critical importance in the transmembrane protein deficient forms of NCL. With a lack of mechanism-based therapies, we run the risk of cataloguing a series of different events, and then try to block them and determine if this affords any benefit [e.g. 47,48,83]. Instead a more basic understanding of which of the different cell types within the NCL brain are affected, and how their interactions are compromised is needed. This should not, however, be limited to the interactions between neurons, or how the timing of disease pathology may track along different pathways. Rather, it should also encompass those functionally crucial interactions between neurons and different classes of glia, between these glial cell types, and the potential influence of the adaptive immune system.

Much information has emerged from, and continues to be uncovered in, a series of different disease models [6,23], which range from simple cellular organisms, to small vertebrate, mouse and large animal models of NCL. Perhaps more overlooked is the invaluable resource of human autopsy specimens and what they can reveal [1,77,98], bearing in mind their scarcity and that they can only inform about disease end-stage.

This review article will look at some of the main pathological features that have been reported across the different forms of NCL, and consider how these have informed our understanding of these disorders.

2. Obtaining a new perspective of NCL pathogenesis via animal models

In addition to aiding diagnosis, one of the major benefits of identifying disease causing genes has been the subsequent ability to generate animal models in which these genes have been mutated or to identify spontaneous mutants that carry similar gene defects [e.g. 20,21,29,35, 44,46,60,79,86]. Each of these species has its benefits and drawbacks as disease models, but the most widely utilised models are genetically engineered mutant mice [84], with models now existing for the vast majority of disease sub-forms [23]. Particularly relevant for addressing the issues of how to deliver therapies, several different larger animal models of disease have also been identified [22], the most commonly used being dogs and sheep [e.g. 3,4,10,27,38,57,94]. As technology advances, it is now possible to generate models in species such as pigs [24,25], which are likely to prove especially valuable because of the perceived closeness of porcine and human physiology.

A significant advance was crossing the different mouse models of NCL onto a common strain background, which made it possible to make comparisons between these models and address key issues about the relative staging of disease progression [15,17,71,84]. Ultimately the disease end point is a brain that is atrophied, and contains many fewer neurons, all of which contain large amounts of storage material [77,98]. This is invariably accompanied by profound astrocytosis and microglial activation, and there may also be a relatively low level infiltration of lymphocytes into the NCL brain [33,52]. While these general pathological themes hold true across most, if not all forms of NCL, their relative severity and timing (or even whether they occur at all) can differ markedly between mouse models.

While the mouse models of earlier onset forms of NCL generally display more pronounced phenotypes, there are certain anomalies such as the relative severity of Tpp1/Cln2 deficient mice compared to Ppt1/Cln1 deficient mice [35,86]. These may be due to technical issues encountered in generating this mouse model of Cln2 disease, but may also reflect an as yet unidentified species-specific consequence of Tpp1 deficiency in mice. It is also apparent that the extent of brain pathology is much less pronounced in mouse models, than in a larger animal model of the same form of NCL, or in the human condition itself [e.g. 63,68,69,98]. This may be due to the fact that mice do not live long enough to develop the full range of human pathology. However, in general, it seems to be that the larger and more complicated a brain is, the more severely affected it will be by this disease.

3. New lessons from mouse models of NCL

Despite such apparent limitations, much valuable information has been gained from mouse models of NCL [reviewed in 6,23,84], not just about pathogenesis [17,61,71], but also in testing experimental therapies [reviewed in 65]. Indeed, analysing these mice has given us a series of novel perspectives about the relationship between the events that occur during disease progression. These include the extent to which specific brain regions, pathways and cell types are affected, and how this may vary between forms of NCL. This is not necessarily surprising given the widely different nature, and probable intracellular location, of the gene products that are deficient in these disorders [12,45]. Indeed, perhaps more surprising is that these disorders resemble each other as much as they do. Another key concept to emerge from studying these mouse models is that several long-standing theories about the pathogenesis of the NCLs may not hold true upon closer examination [15].

4. Selective neuron loss in the NCL brain

Perhaps the first of these novel insights was the discovery that although the brain is indeed severely impacted by the time of death, albeit to different extents in disease subtypes, as reviewed in this article, the nature of neuron loss is actually rather selective in the earlier stages of disease. The initial observations of such selective vulnerability focused upon populations of hippocampal and cortical interneurons [e.g. 5,16, 63,69,75,76], but were subsequently extended to the cerebellum [55, 104], and thalamocortical system (see below), and it is likely that other examples will be found. However, just taking the first example, comparing interneuron loss across mouse models reveals a bewildering array of specific effects upon interneurons that express different calcium binding proteins or neuropeptides, or those that are located in different hippocampal subfields or cortical regions [5,49,63,69,75,76, 103]. Despite any mechanistic evidence for why these subpopulations of neurons are specifically affected, such marked differences highlight that while a pathological feature may be broadly shared across NCL subtypes, it is too simplistic to assume that these events occur in the same fashion.

Another surprising observation made in mouse models was the pathological targeting of the thalamus relatively early in disease progression. This phenotype so far holds true across nearly all forms of NCL [e.g. 39,49,63,73,76], with the loss of thalamic relay neurons preceding neurodegeneration in the corresponding region of the cortex to which it projects. Certainly, within any given mouse model the death of neurons that relay different modalities of information to the cortex does not occur at the same time, but is staged at different points of disease progression [e.g. 39]. Nevertheless, the relative timing of these events within the thalamocortical system also varies between forms of NCL [reviewed in 71], and while these may at first glance appear to be relatively minor variations, they reveal further evidence for different consequences of mutations in these genes at a cellular level. A starker example of the contrasting effects of gene mutations comes from *Cln5* deficient mice in which the sequence of neuron loss is reversed [100], and apparently occurs in the cortex before the thalamus, a feature that is so far unique amongst mouse models.

5. Pathway dependent pathology and synaptopathy

The progressive staging of pathological events in the thalamocortical system highlighted the possible importance of connectivity in determining the order in which neuron populations are lost in the NCL brain. The concept of neurodegeneration spreading along pathways in either an anterograde or retrograde direction is not a new one, but has

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