Molecular Psychiatry (2002) 7, 434–436

www.nature.com/mp

NEWS & COMMENTARY

Neuronal ceroid lipofuscinosis/Batten disease: the lysosomal proteinoses

Molecular Psychiatry (2002) **7,** 434–436. doi:10.1038/sj.mp.4001127

Excessive protein accumulation is a recurring theme in the hereditary neurodegenerations. The neuronal ceroid lipofuscinoses (Batten disease) provide another example—with the twist that the lysosomal compartment is the site of abnormal protein storage, due to defects in lysosomal proteases or related enzymes.

The abnormal accumulation of specific proteins in neurons has emerged as a consistent theme in neurodegenerative disorders: β-APP in Alzheimer disease, α-synuclein in familial Parkinson's disease and Lewy body dementia, and polyglutamine in Huntingdon disease and certain spinocerebellar atrophies. In each case, for mysterious reasons, the failure to metabolize a particular protein leads to selective loss of a population of cells in the brain, with devastating clinical consequences. The obvious conclusion is that the neuron, a post-mitotic cell, is particularly vulnerable to this type of insult.

Long before these recent developments, classical hereditary neurodegenerative diseases related to the abnormal accumulation of other cell components (for example, various glycolipids) have been well characterized. These disorders comprise the classical lysosomal storage diseases such as Tay–Sachs disease, Krabbe disease, and metachromatic leukodystrophy, to name a few. Until very recently, no disorders related to defective lysosomal *proteolysis* had been described, but this situation has changed now that the underlying basis for an older group of disorders, the neuronal ceroid lipofuscinoses (NCLs, also known as Batten disease) have been uncovered.

The NCLs are a group of neurodegenerative disorders that usually begin in childhood, and are characterized by blindness, myoclonic seizures, progressive motor disturbances, and dementia.[1] Of note, many affected older children exhibit troubling psychiatric symptoms early in the course of the disease. Three major forms comprise infantile, late infantile, and juvenile-onset disease and are caused by mutations in the CLN1, CLN2, and CLN3 genes, respectively. Very recently, two more genes, CLN5 and CLN6,[2,3] responsible for variant late-infantile forms have been cloned, and a sixth gene, CLN8, was recognized as underlying a form of progressive myoclonic epilepsy. The common characteristic in all of these disorders is a striking accumulation of autofluorescent storage material in all tissues, but especially the central nervous system. The storage material appears in membrane-delimited organelles with the structure and staining characteristics of 'residual bodies,' the presumed end-result of lysosomal degradation in the cell.

The classification of the NCLs based on age of presentation has been blurred with the finding of less severe mutations in CLN1 and CLN2 that overlap clinically with CLN3. An extreme example came to light recently in the description of two sisters in their thirties who suffered from depression and dysthymic disorder for nearly 10 years before the progressive nature of the illness became apparent.[4] A brain biopsy in one sister revealed granular osmiophilic deposits characteristic of PPT1 deficiency, which was confirmed by enzyme assay. As in other neurodegenerative disorders in which protein accumulation is a factor, there was a very long period of subclinical disease, followed by an abrupt deterioration. It is almost as if a reservoir was being filled, which finally reached the top and spilled over, with devastating clinical consequences.

What have we learned about these disorders from knowledge of the underlying genes, and what is the evidence for a primary defect in proteolysis? The most important clues have come from the known functions of three of the NCL genes. To start with the most straightforward example, CLN2 (which underlies late-infantile NCL) encodes tripeptidyl amino peptidase, or TPP-I. This enzyme specifically cleaves tripeptides from the amino-termini of partially unfolded proteins—it is literally, an 'enzyme that counts'.[5] TPP-1/CLN2 is the first example of the involvement of a lysosomal protease in a neurodegenerative storage disease. The major intact protein that is identified in the storage material in TPP-I deficiency is subunit *c* of mitochondrial ATP synthase. Subunit *c* is a very hydrophobic protein and a major component of mitochondria; it may be significant that partially digested mitochondria are frequently found as a normal component of residual bodies in cells. *In vitro* evidence suggests that TPP-I may be required to initiate degradation of this very hydrophobic protein. Following on the heels of that discovery, it was shown that a sheep model of NCL, congenital ovine NCL, has an underlying defect in the lysosomal protease cathepsin D.[6] These animals are born weak, with microcephaly and progressive neurological impairment and die shortly

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after birth. (Interestingly, the cathepsin D knockout mouse, which was originally used to demonstrate the role of cathepsin D in antigen processing, was upon further examination found to suffer from a similar neurodegenerative fate.[7] Although cathepsin D deficiency is unknown in humans, the sheep model provides a second example showing that lysosomal protease deficiency can lead to the formation of residual bodies and autofluorescent storage that is characteristic of the NCLs. Finally, CLN1 (infantile Batten disease) is caused by inherited recessive mutations in a gene encoding lysosomal palmitoyl-protein thioesterase. The function of this enzyme is to remove fatty acids from a variety of fatty acylated proteins, where the fatty acid is covalently linked to cysteine residues. Small peptides that contain fatty acid and cysteine accumulate in the disorder, providing a third example of accumulation of proteinaceous material in NCL, but in this instance, fatty acylated peptides are involved.[8]

The other genes that cause forms of NCL (CLN3, 5, 6 and 8) unfortunately provide little information from their sequence as to function. The CLN3 protein, referred to as battenin, is an intrinsic membrane protein, with multiple membrane spanning regions, that bears no homology to known proteins, but its lysosomal localization clearly reinforces the classification of the NCLs as lysosomal storage disorders.

The NCLs would therefore seem fundamentally to be lysosomal proteinoses. That said, what progress might we expect in these disorders in the future? In this light, we point to several recent mouse models of the NCLs. A CLN1/PPT1 mouse model was recently described[9] that faithfully reproduces the key features of the human disorder. These mice develop spasticity at 4–5 months of age, followed by ataxia, myoclonus, generalized seizures, and death by 10 months of age. In brain regions such as the cerebellum where there is normally strong expression of PPT1 ((Figure 1, panel a), in PPT1-deficient mice there is striking accumulation of strongly autofluorescent storage material (panel b). At the light microscopic level, neuronal dropout of Purkinje cells is severe (panel c) and many of the remaining cells are apoptotic (panel d). Of note, deletion of a related gene, PPT2, caused a similar phenotype, with a slower accumulation of lipofuscin. PPT2 is a second lysosomal thioesterase that hydrolyzes thioester-linked fatty acids but its endogenous substrate(s) are unknown. Mouse models have also been created for juvenile Batten (CLN3) disease.[10,11] Two naturally occurring NCL models, the *mnd* mouse and *nclf* mouse, are known to correspond to the human CLN8 and CLN6 genes, respectively.[2,3]

Mouse models will surely facilitate a better understanding and the development of treatments for these disorders. What forms of therapy can be anticipated? A substrate depletion strategy employing the drug cysteamine has shown promise in cell culture experiments.[12] Cysteamine has a relatively long history for treatment of the lysosomal storage disorder cystinosis and can be tested expeditiously in mice and in humans with the infantile form of NCL. Enzyme replacement therapy (for CLN1 and CLN2 disease) and gene therapy experiments for several of these NCLs are underway. Progress in the NCLs, the lysosomal proteinoses, has been very rapid and there is cause for hope that new treatments for these disorders will ultimately be forthcoming.

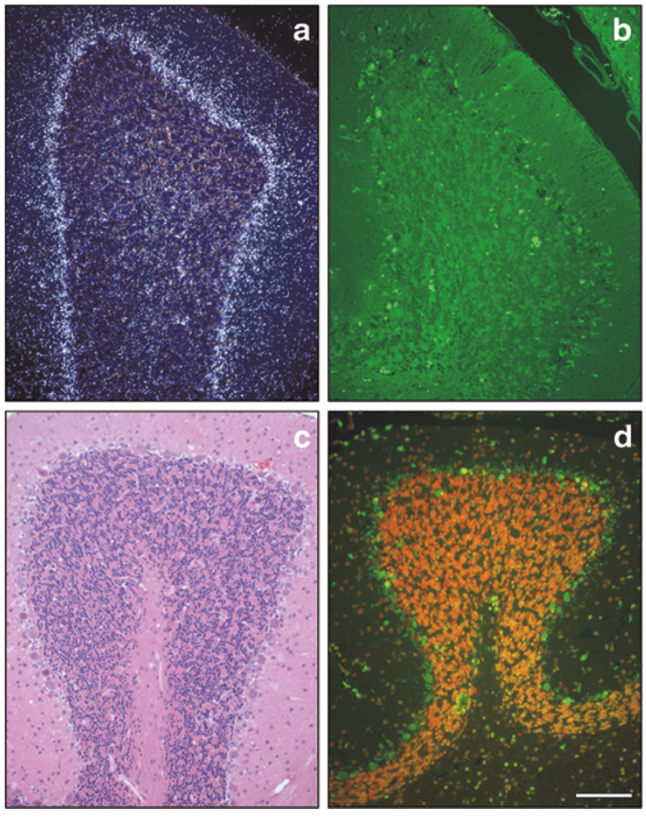

Figure 1 Consequences of PPT1/CLN1 deficiency in the Purkinje layer of mouse cerebellum. (a) RNA *in situ* hybridization of normal mouse cerebellum shows intense white PPT1 signal in Purkinje neurons. (b) Autofluorescence (bright green) in Purkinje layer of a 6 month-old PPT1 knockout mouse. Autofluorescent deposits are the defining characteristic of the neuronal ceroid lipofuscinoses. (c) Ballooning degeneration and neuronal dropout in the Purkinje layer of a PPT1 knockout mouse brain. (d) Apoptotic neurons as revealed by TUNEL staining (yellow). Nuclei are counterstained with propidium iodide (red). Bar, 100 μm. Photomicrographs courtesy of John Shelton, Division of Cardiology, Department of Internal Medicine, UT Southwestern Medical Center. Bar, 100 μm.

Acknowledgements

This work is supported by the National Institute for Neurological Disorders and Stroke (NS36867 and NS35323) and the Batten Disease Support and Research Association.

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