Targeted Disruption of the Cln3 Gene Provides a Mouse Model for Batten Disease

Hannah M. Mitchison,* David J. Bernard,† Nicholas D. E. Greene,* Jonathan D. Cooper,‡ Mohammed A. Junaid,§ Raju K. Pullarkat,§ Nanneke de Vos,¶ Martijn H. Breuning,¶ Jennie W. Owens,‖ William C. Mobley,‡ R. Mark Gardiner,† Brian D. Lake,** Peter E. M. Taschner,¶ and Robert L. Nussbaum†

*Department of Paediatrics, Royal Free and University College London Medical School, Rayne Institute, University Street, London, WC1E 6JJ, United Kingdom; †Genetic Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland 20892-4472; ‡Department of Neurology and Neurological Sciences and the Program in Neuroscience, Stanford University, Stanford, California 94305-5489; §New York State Institute for Basic Research in Developmental Disabilities, Staten Island 10314, New York; ¶Department of Genetics, Section of Human Genetics, Sylveus Laboratories, Leiden University, P.O. Box 9503, 2300 RA Leiden, The Netherlands; ‖Veterinary Resource Program, Office of Research Services, National Institutes of Health, Bethesda, Maryland 20892-4472; **Department of Histopathology, Great Ormond Street Hospital for Children, London WC1N 3JH, United Kingdom

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Batten disease, a degenerative neurological disorder with juvenile onset, is the most common form of the neuronal ceroid lipofuscinoses. Mutations in the CLN3 gene cause Batten disease. To facilitate studies of Batten disease pathogenesis and treatment, a murine model was created by targeted disruption of the Cln3 gene. Mice homozygous for the disrupted Cln3 allele had a neuronal storage disorder resembling that seen in Batten disease patients: there was widespread and progressive intracellular accumulation of autofluorescent material that by EM displayed a multilamellar rectilinear/fingerprint appearance. Inclusions contained subunit c of mitochondrial ATP synthase. Mutant animals also showed neuropathological abnormalities with loss of certain cortical interneurons and hypertrophy of many interneuron populations in the hippocampus. Finally, as is true in Batten disease patients, there was increased activity in the brain of the lysosomal protease Cln2/TPP-1. Our findings are evidence that the Cln3-deficient mouse provides a valuable model for studying Batten disease.

INTRODUCTION

The neuronal ceroid lipofuscinoses (NCL) are a group of autosomal recessive disorders that comprise the most common neurodegenerative diseases of childhood, with an incidence of up to 1:12,500 (Santavuori, 1998). The clinical course is progressive and is marked by blindness, seizures, psychomotor deficits and dementia leading to a vegetative state and death in early adulthood (reviewed in Santavuori, 1998; Rapola, 1993; Goebel et al., 1999). Widespread loss of neurons in the neural retina and central nervous system (CNS), predominantly the cortex and cerebellum, account for the neurological symptoms. No other organs appear to be clinically affected. The juvenile onset form of NCL, referred to as Batten disease (JNCL), is caused by mutations in the CLN3 gene (International Batten Disease Consortium, 1995). The CLN3 gene encodes a 438 amino acid predicted transmembrane protein (International Batten Disease Consortium, 1995; Janes et...
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newly synthesised proteins to the lysosome (Sleat

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may reflect increased synthesis, a common feature of

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level of lysosomal proteins is associated with a general

like dense bodies in neurons (Ivy

Batten disease, resulting in accumulation of lipofuscin-

Finally, treatment with inhibitors of lysosomal prote-

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METHODS

Construction of Targeting Vector

The Cln3-targeting vector was constructed as out-

lined previously (Greene et al., 1999). The insert from

mouse Cln3 cDNA clone pMNCL (Taschner et al., 1997)

was tested by Southern blot hybridization to verify it

was single copy sequence. This fragment was used to

probe a 129/Sv mouse genomic cosmid library (van

Ree et al., 1994). One cosmid, mcos6, was found to

contain the complete Cln3 gene by Southern hybridiza-

tion, using CLN3 cDNA fragments as probes, by PCR

using exon-specific primers, and by partial sequence

analysis. A 9-kb genomic HindIII fragment containing

exons 1–8 of Cln3 was isolated from mcos6 and cloned

into the pBluescript II SK+ HindIII site (Stratagene,

CA). A SacII–EcoRI fragment of this clone containing

Cln3 exon 1 (including the start codon) and exons 2–6

was replaced with a 1.9-kb HindIII fragment contain-

ing a PGK promoter–neomycin resistance–PGK-polyA

signal cassette cloned in the reverse orientation. This

was achieved by end-filling the SacII, EcoRI, and

HindIII sites, followed by blunt-end ligation. A 2740-bp

cassette containing the herpesvirus thymidine kinase

gene under control of the PGK promoter was digested

from the pPNT vector (Tybulewicz et al., 1991) with

EcoRI and HindIII and endfilled. This fragment was

inserted at an endfilled HindIII site at the 5′ end of the

9-kb Cln3 fragment for negative selection. An XhoI site

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disease is an important step toward elucidating underly-

ing disease mechanism and testing potential treat-

ment strategies. Although NCLs occur naturally in a

range of animal species (Goebel et al., 1999; Jolly, 1995),

there is no model in which the disease is caused by

mutations in CLN3. By targeted disruption of the mouse

Cln3 gene in embryonic stem cells, we generated

mice with a Cln3 null allele. Progressive accumu-

lation of autofluorescent material with the staining and

ultrastructural characteristics of the material stored in

Batten disease patients was detected in mice homozy-

gous for the disrupted Cln3 allele. In addition, there

were neuropathological abnormalities involving the

cortex and hippocampus. Cln2 protease activity in the

brain was significantly elevated, implying that a corre-

lation with human disease also exists at the biochemi-

cal level. We conclude that because Cln3-deficient mice

recapitulate several neuropathological features seen in

human patients, they provide a model for the study of

Batten disease.

al., 1996) whose function is unknown. On the basis of

structural predictions, it has been suggested that CLN3

could be a transporter (Pearce et al., 1999), and there is

potential functional homology to nucleotide-sugar

transporters at residues 193–214.

The pathogenesis of Batten disease is unknown, but

a compelling case can be made for the involvement of

lysosomes. First, a key diagnostic feature of Batten

disease is the intracellular accumulation of autofluores-

cent material within lysosomes. Stored material is

present in neurons as well as in other cell types

(Rapola, 1993; Goebel et al., 1999; Lake, 1997). This

material resembles lipofuscin, a substance found in

normal aging brains of humans and mice (Mann et al.,

1978; Sekhon & Maxwell, 1974). However, the deposits

in JNCL are unique in that they have a characteristic

multilamellar “fingerprint” ultrastructure and contain

subunit c of the mitochondrial ATP synthase as their

major protein component (Hall et al., 1991; Palmer et

al., 1992). Second, potential dileucine and tyrosine-

based (residues 52–55) lysosomal targeting signals are

present in the CLN3 protein (White et al., 1992; Hun-

ziker & Fumey, 1999). The presence of such signals is

consistent with the recently reported localization of

CLN3 in lysosomes (Järvelä et al., 1998, 1999). Third,

the clinical features and composition of storage mate-

rial in Batten disease are similar to another disorder,

classical late infantile NCL (CLN2), which is known to

be caused by mutations in a lysosomal pepstatin-

insensitive tripeptidyl peptidase I (CLN2 protease/

TPP-1) (Sleat et al., 1997; Vines & Warburton, 1999).

Fourth, the brains of Batten disease patients show a

significant increase in the level of glycoproteins contain-

ing mannose-6-phosphate, a modification that directs

newly synthesised proteins to the lysosome (Sleat et al.,

1998; Junaid & Pullarkat, 1999). This increase in the

level of lysosomal proteins is associated with a general

elevation in the activity of lysosomal enzymes, which

may reflect increased synthesis, a common feature of

other lysosomal storage diseases in which one enzyme

is defective (Burditt et al., 1980; Young et al., 1997).

Finally, treatment with inhibitors of lysosomal prote-

ases recreates certain features of the pathology of

Batten disease, resulting in accumulation of lipofuscin-

like dense bodies in neurons (Ivy et al., 1984). This

evidence suggests that dysfunction of one or more

aspects of lysosomal biology contributes to Batten

disease pathogenesis.

The development of an animal model that recapitu-

lates the clinical and pathological features of Batten

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in the pBluescript polylinker creates a unique vector linearization site. The targeting vector comprised 4 kb of genomic sequence upstream and 2 kb downstream of the PGKneo cassette.

**Gene Targeting in Embryonic Stem Cells**

The targeting construct was linearized with XhoI and electroporated into 129/Sv TC1 ES cells as previously described (Deng et al., 1996). Targeting was identified in two of 140 G418- and FIAU-resistant colonies by EcoRV Southern blot analysis using a probe located outside the targeted region of homologous recombination (probe C). Positive cell lines were confirmed by EcoRV genomic Southern blot using external probe A and internal probe B (see Fig. 1).

**Generation of Cln3 Null Mice**

Two correctly targeted ES cell lines were used to generate chimeras by injection into C57BL/6 blastocysts followed by transfer into pseudopregnant recipient NIH Black Swiss females, using standard techniques (Hogan et al., 1994). One ES cell line produced chimeras that showed germline transmission as indicated by the agouti coat color of offspring following breeding to NIH Black Swiss females. F1 offspring were genotyped by EcoRV genomic Southern blot using external probe A and internal probe B (see Fig. 1).

**RT-PCR**

Brain and kidney samples were collected into liquid nitrogen and total RNA isolated with RNAzol (Biogenesis Ltd., UK). RT-PCR of total RNA was performed using the Superscript kit (Life Technologies, Inc.), according to the manufacturer’s instructions with Neomycin primers 1–5’ (5’-AGAGGCTATT-CGGCTATGACTG-3’) and 2–3’ (5’-TTCGTCCAGATCATCCCTGATC-3’) or Cln3 primers M7F (5’-ACCCACCTGTCCAGACTTT-3’) and M6R (5’-CACTCCGAATCTCCACCGA-3’). M7F and M6R amplify within the coding region of Cln3, across an intron, such that DNA contamination can be excluded based on size difference of the relevant PCR products.
rons in the hippocampus and cortex as described previously (Cooper et al., 1999).

**Unbiased Estimates of Regional Volume**

Unbiased estimates of the volume of the hippocampus and adjacent cortical areas were made using Cavalieri’s method (Cavalieri, 1966), by counting the number of points of a randomly superimposed sampling grid which fell over each structure in a one-in-six series of Nissl-stained coronal sections as described previously (Cooper et al., 1999).

**Measurements of Detectable Neuronal Number and Cross-Sectional Area**

A one-in-six series of sections through the hippocampal formation and cortical mantle of each brain was stained to reveal the presence of neurons expressing parvalbumin (PV), calbindin (Cb), or somatostatin-14 (SOM). Counts were made of detectable PV-expressing neurons in layer II and IV of the entorhinal cortex and PV-, Cb-, and SOM-positive neurons in the hippocampal formation. Counts were expressed as the number of detectable neurons per section and corrected by the method of Abercrombie (Abercrombie, 1946). Measurements of neuronal cross-sectional area were made in the same sections. All methods used as described previously (Cooper et al., 1999).

**Cln2 Protease Activity**

Brains were collected in liquid nitrogen and frozen until use. The Cln2 protease enzyme activity was measured as described previously (Junaid & Pullarkat, 1999; Junaid et al., 1999).

**RESULTS**

**Gene Targeting of Cln3**

To inactivate Cln3, exons 2–6 and most of exon 1, including the start codon, were deleted in TC1 embryonic stem cells (Figs. 1A and 1B) by replacement with a neomycin resistance gene transcribed in reverse orientation from a mouse PGK promoter, as previously described (Greene et al., 1999). Chimeras established from these cells were used to generate both 129/Sv inbred and 129/Sv × Black Swiss outbred lines; the outbred line was used for subsequent analysis (Fig. 1B). Intercrosses between F1 heterozygotes produce viable F2 offspring in normal Mendelian ratios: wild-type \((n = 27, 25\%)\), heterozygote \((n = 59, 55\%)\), and homozygous offspring \((n = 22, 20\%)\). RT-PCR confirmed the absence of Cln3 transcripts in homozygous mutant mice (Fig. 1C). Therefore, although the gene is known to exhibit widespread low-level expression during embryonic development (N. D. E. Greene and H. M. Mitchison, unpublished data), Cln3 function is not essential for development of the embryo. Cln3 \(–/–\) mice were viable and fertile and by 12 months of age did not exhibit obvious clinical signs.

**Cln3 \(–/–\) Mice Exhibited Accumulation of Intracellular Storage Material**

In spite of the absence of evident clinical disease, morphological studies showed that Cln3 \(–/–\) mice exhibited several of the features of Batten disease. We observed a marked accumulation of storage material in neuronal cell bodies in the brains of Cln3 \(–/–\) (Fig. 2). Autofluorescent cytoplasmic inclusions were detected in Cln3 \(–/–\) mice at 3 months of age and analysis up to 12 months of age showed that there was an increasing amount of material, indicating that the storage was progressive (Figs. 2A–2D). Storage levels at 5 months of age have also previously been described (Greene et al., 1999). Stored material fluoresced over a wide range of excitatory and barrier filter combinations. It appeared to be typically granular and was distributed widely throughout the cytoplasm, always sparing the nucleus. The composition of the stored material also appeared to be similar to that in human patients, because it stained positively with the lipid stains Luxol fast blue (Figs. 2E and 2F) and Sudan black (data not shown). Significantly, the material stored in Cln3 \(–/–\) mice did contain subunit c of the mitochondrial ATP synthase, a major component of storage bodies in Batten disease patients (Figs. 2G and 2H).

Autofluorescent material was distributed widely in the brain and was found in both neurons and glial cells. Storage was particularly prominent in neurons of the cortex, hippocampus, basal ganglia, and reticular formation of the brainstem. Within the hippocampus the distribution was very similar to that seen in the mnd mouse, which is a model of another form of NCL (Cooper et al., 1999; Ranta et al., 1999), with prominent accumulation in interneurons of the hilar formation, stratum oriens, stratum radiatum, and in interneurons dispersed among the principal cell layers (CA1-3). A representative comparison of the hippocampus of a wild-type and a mutant animal at 7 months of age is shown in (Figs. 3A and 3B). The superior resolution
afforded by confocal microscopy permitted visualization of sparse deposits of lipopigment in the cytoplasm of granule neurons of the dentate gyrus (Fig. 3B). Heterozygous mice were indistinguishable from wild-type mice (not shown).

Under electron microscopy, storage bodies showed a multilamellar ultrastructure that is very reminiscent of the rectilinear/fingerprint profile seen in Batten disease (Rapola, 1993; Goebel et al., 1999; Lake, 1997). The number of individual storage bodies within cells in-

FIG. 1. Generation of Cln3-deficient mice by homologous recombination. (A) Structure of the wild-type allele, the targeting vector, and the predicted targeted Cln3 locus. The external and internal probes used for detection of targeted ES cells are indicated below the wild-type allele (probe A, B, C) and the relevant restriction sites are shown (R1, EcoRI; RV, EcoRV; H, HindIII; S, SacII). Figure drawn to scale. (B) Southern blot of EcoRV-digested genomic DNA. Homologous recombination results in the reduction of a wild-type 9.5-kb allele (lane 1) to a 5.5-kb allele (lane 2) in targeted ES cells (probe C). Representative samples from the offspring of an intercross between Cln3 heterozygous mice are included in lanes 3–5. Hybridization with probe B indicates heterozygote (lane 3), homozygous −/− (lane 4), and wild-type (lane 5) genotypes. (C) mRNA expression analysis of kidney (lanes 1 and 2) and brain (lanes 3 and 4) by RT-PCR indicates absence of Cln3 exons 1 and 2 and expression of the neo cassette in homozygous Cln3 −/− mice (lanes 2 and 4). Wild-type controls in lanes 1 and 3.
FIG. 3. Confocal and electron microscopy of storage material. (A, B) Unstained sections of hippocampus from 7-month-old mice visualized by UV confocal microscopy shows autofluorescent intracellular inclusions in the dentate gyrus (dg) and hilus (hi) in mutant (B) compared to wild-type (A). Magnification ×300. (C, D) Representative images of storage material from 12-month-old mutants visualized by electron microscopy illustrate the multilamellar electron-dense ultrastructure of the inclusion bodies. Magnification, ×22,400 (C) and ×33,570 (D).

FIG. 2. Histological and immunochemical analysis of Cln3 −/− mice. (A–D) Under UV light, autofluorescent inclusions (arrows in B, D) are detected in unstained sections of cortex from homozygous mutant (B, D) mice at 5 (A, B) and 12 (C, D) months of age compared to a low background autofluorescence in age-matched controls (A, C). Storage material in 8-month-old mutants (E, H) also stains positively with Luxol fast blue (E, F) and antisera to subunit c of the mitochondrial ATP synthase (G, H), compared to background levels in age-matched controls (E, G). Representative sections from hippocampus are shown; storage bodies are indicated by arrows. Magnification, ×1000 (A–D), ×1200 (E–H).
increased with age and these usually had a compact multi-layered structure containing a number of more electron-dense areas (Fig. 3C). Later in the disease course, larger and less tightly packed bodies were observed occupying the majority of the cell soma (Fig. 3D). In the wild-type littermate controls, an occasional deposit of autofluorescent lipofuscin resulted in a small increase in the background levels of autofluorescence (Also seen in Figs. 2A and 2D). However, these naturally occurring deposits were simple dense structures in secondary lysosomes (not shown) (Mann et al., 1978; Sekhon & Maxwell, 1974). They were rare and readily distinguished from the inclusion bodies seen in Cln3−/− mice.

**Disruption of Cln3 Caused Hypertrophy and Loss of Specific Populations of Interneurons**

We examined the brains of Cln3−/− mice at 7 months of age in order to determine, whether accumulation of autofluorescent material was associated with other neuropathological changes. There was a small reduction in total brain mass in mice homozygous for the disrupted Cln3 allele (Table 1). However, the change was not statistically significant. Unbiased volumetric analysis of the neocortex revealed a decrease that approached statistically significance. There was no reduction in the volume of the hippocampal formation in Cln3−/− mice (Table 1).

We examined subpopulations of neurons containing autofluorescent accumulated storage material. Apparent loss and hypertrophy of remaining interneurons has been reported in the mnd mouse (Cooper et al., 1999), suggesting that these neuronal populations may also be susceptible in Cln3−/− animals. Therefore, we stained sections through the hippocampal formation and cortex for immunohistochemical markers of interneuronal phenotype. We examined interneuronal populations that stained positive for the calcium-binding protein parvalbumin (PV) in the entorhinal cortex, and subpopulations that stained positive for PV or another calcium-binding protein calbindin (Cb) or the modulatory neuropeptide somatostatin (SOM) in the hippocampal formation.

Significantly fewer (P < 0.05) PV-positive interneurons were detected in layers II and IV of the cortex in Cln3−/− mice as compared with controls (Fig. 4A). Measurements of cross-sectional area indicated that persisting PV-positive neurons in the entorhinal cortex of these mice were of the same size as controls (Figs. 4B and 4C). Though we found mild atrophy of these PV-positive interneurons in the entorhinal cortex of heterozygotes, the change was quite small (Figs. 4B and 4C).

The most significant change in the hippocampus was the hypertrophy of most interneuronal populations examined. The changes were most marked for SOM- and Cb-positive interneurons; in these populations the increases were all statistically significant and in some cases the neuronal areas were increased by almost 25% over control values (Fig. 5B). The population-wide nature of changes in cell size in Cln3−/− mice is clearly seen by comparison of cell-size distribution histograms across different genotypes. Representative examples (Figs. 5C and 5D) show the typical shift of the whole population of cells towards an increase in area. While in heterozygous mice atrophy of PV-positive interneurons was observed, the changes were relatively small (Fig. 5B). Analysis of hippocampal interneuronal number showed a consistent, although not statistically significant, trend towards reduced number of SOM- and Cb-positive neurons in most subregions of Cln3−/− (Fig. 5A). There were no significant changes in the number of PV-positive interneurons. Taken together, our findings point to specific and significant changes in the number and size of certain interneuron populations in Cln3−/− mice.

**Cln2 Protease Activity Is Elevated in Cln3−/− Mice**

To further compare the phenotype of Cln3−/− mice with Batten disease patients we measured the activity of murine Cln2 protease, one of the lysosomal enzymes known to have elevated activity in Batten disease patients (Sleat et al., 1998; Junaid &
Pullarkat, 1999). In parallel with human patients, Cln2 protease activity was significantly elevated in the brains of Cln3 −/− mice compared to wild-type littermates, while heterozygotes are unaffected (Fig. 6). At 5 months of age the activity in Cln3 −/− animals was approximately doubled compared to controls. Similar increases have reported in Batten disease patients. As with accumulation of storage material, this phenotype appeared to be progressive in that it was more pronounced at 5 months than 2 months of age. No increase was seen in heterozygotes (Fig. 6).

**DISCUSSION**

Given that the Cln3 gene has 82% sequence identity and 85% amino acid similarity with human CLN3 (Lee et al., 1996), we hypothesized that Cln3 −/− mice would show many of the pathological and biochemical changes found in human Batten disease patients. We have created mice deficient in the Cln3 gene by gene targeting. Histological analysis reveals progressive cellular abnormalities that are characteristic of Batten disease pathology. There is widespread accumulation of autofluorescent material contained in storage bodies that stain positively for subunit c of the mitochondrial ATP synthase and have a multilamellar ultrastructure. Moreover, Cln3 −/− mice demonstrate an elevated activity of Cln2 protease as is observed in Batten disease patients (Sleat et al., 1998; Junaid & Pullarkat, 1999).

Despite the cellular pathology that develops in Cln3 −/− mice, they did not develop obvious clinical symptoms by 12 months of age. The lack of overt neurological signs is likely to be due to a pathologic process that requires a longer interval to produce symptoms or due to the lack of involvement of neuronal populations whose dysfunction is readily detected or both. Continued observation over time will determine whether the development of neuronal pathology is sufficient to cause clinical symptoms prior to age-related death. It is noteworthy that Cln3 −/− mice are less severely affected than the autosomal recessive NCL mouse models, mnd (motor neuron degeneration) and nclf (neuronal ceroid lipofuscinoses), which exhibit progressive motor dysfunction from 4 and 8 months of age, respectively (Messer & Flaherty, 1986; Bronson et al., 1993, 1998; Pardo et al., 1994). The motor deficits in mnd and nclf are preceded by accumulation of NCL-like storage material from just a few weeks of age followed by retinal atrophy from approximately 4 months of age (Bronson et al., 1998; Chang et al., 1994). These observations suggest that the presence of stor-
FIG. 5. Neuronal number and area in hippocampus of mice at 7 months of age. (A) Abercrombie-corrected counts of detectable interneuronal number per coronal section through hippocampus (5 sections per animal). (B) Mean cross-sectional area of interneuron subpopulations (number of neurons, n = 120–150, except SOM- and PV-positive radiatum, n = 60–73. *t test, * P < 0.0005; ** P < 0.05 vs wild-type. # P < 0.0005; ## P < 0.05 vs Cln3−/−). (C, D) Representative plots of cell size distribution for neuronal areas of (C) SOM-positive neurons in oriens and (D) Cb-positive neurons in radiatum.
months of age but to a lesser extent. Ase activity is also elevated (**
months of age. Carriers are indistinguishable from wild-type. Prote-
subtype (Goebel et al.,
somal map location (Lee
symptoms do not develop until 4–7 years of age. In accordance with the milder
phenotype, the CNS of
Cln3 mouse is that disruption of Cln3/CLN3 influences neurons whose dysfunction is less evident clinically than those affected CLN6 or CLN8.

Though the cellular changes in Cln3 –/– mice were less severe than in mnd and nclf mice, we did observe significant loss of one subpopulation of neurons (PV-positive) in the entorhinal cortex, which is also reflected in the hippocampus, in addition to a strong trend toward reduced numbers of SOM-positive interneurons in the hippocampus. It is not yet clear whether the apparent loss of interneurons is due to cell death or the down-regulation of normally expressed phenotypic markers (Cooper et al., 1999). There was also significant hypertrophy of many subpopulations of interneurons, indicating dysregulation of cell volume. In contrast, cortical interneurons, although reduced in number in Cln3 –/– mice, did not exhibit hypertrophy. Distinct effects of Cln3 disruption on different interneuronal populations raise the possibility that different mechanisms may be operative. Since there is progressive cortical and cerebellar atrophy and selective loss of neurons in Batten disease, it will be important to examine the CNS of aged mice for evidence of the progressive development of more significant pathologic changes. Studies of retinal function are currently ongoing in the Cln3 –/– mouse (S. Nusinowitz and H. M. Mitchison, unpublished data).

Histological examination of the CNS of Cln3 –/– mouse revealed several features in common with the other NCL mouse models. In mnd mouse, GABAergic interneurons of the hippocampus and neocortex also exhibit dense accumulations of autofluorescent material in interneurons and loss of staining for certain phenotypic markers together with pronounced hypertrophy of remaining detectable interneurons (Cooper et al., 1999). Further evidence for the pathologic involvement of interneurons has also been found in the nclf mouse (J. D. Cooper, unpublished data). Although strain differences do not permit direct comparisons, it is apparent that GABAergic interneurons are affected in each of these three mouse models of NCL. Inhibitory interneurons in the hippocampus and cortex exert a powerful influence upon excitatory transmission in these brain regions (Freund & Buzsaki, 1996; Singer, 1996). It will be important to examine whether morphological abnormalities in Cln3 –/– mice are reflected by alterations in excitatory transmission or thresholds for seizure activation.

Recent progress in understanding the possible function of the CLN3 protein has come from studies of a S.

![FIG. 6. Cln2 protease activity (nmol/h/mg protein) in brains of Cln3 –/– mice. Cln2 protease activity is significantly elevated (*t test, P < 0.001) in brains of –/– compared to +/+ or +/– mice at 5 months of age. Carriers are indistinguishable from wild-type. Protease activity is also elevated (**) test, P < 0.005) in –/– mice at 2 months of age but to a lesser extent.](image-url)
cerevisiae ortholog, BTN1. BTN1 is not required for viability or for degradation of the mitochondrial ATP synthase (Pearce & Sherman, 1997). The orthologous protein Btn1p localizes to the vacuole, the yeast lysosome equivalent, which is responsible for lysosomal function as well as osmotic regulation (Pearce et al., 1999; Croopnick et al., 1998). Yeast carrying a null allele of BTN1, strain (btn-1Δ), are resistant to ANP (β-α-amino-1-[p-nitrophenyl]-1,3-propanediol) due to an increased ability to acidify the growth medium. This phenotype is associated with a lowered pH in the mutant vacuole early in the growth course and is rescued by introduction of the human CLN3 gene (Pearce & Sherman, 1998), implicating involvement of CLN3 in cellular pH regulation (Pearce et al., 1999). DNA microarray analysis shows that a yeast gene with homology to human and drosophila HOOK1, known to be involved in endocytosis (Kramer & Phistry, 1996, 1999), is upregulated in the btn-1Δ strain, indicating possible impairment of protein trafficking in mutant cells (Pearce et al., 1999). The results in yeast suggest possible pathogenetic mechanisms for Batten disease in mice and humans. Altered lysosomal pH regulation as a result of Cln3 disruption may reduce the activity of lysosomal proteases, with profound effects on the degradation of proteins such as mitochondrial ATP-synthase subunit c within the lysosome, cause abnormal aggregation of proteins, or interfere with intracellular protein trafficking. In addition, loss of Cln3 function may interfere with the regulation of cellular volume. Little is known about the mechanisms underlying this process, but controlled shifts in lysosomal activity represent a means to regulate neuronal cytoplasmic volume.

The targeted disruption of Cln3 provides an animal model for a known NCL-associated gene in humans. Further studies are required to assess subtle behavioral abnormalities and to determine whether clinical symptoms develop in aging mice. However, many of the characteristic features of Batten disease are recapitulated in the Cln3−/− mouse, indicating that it will provide a valuable model system for analyzing disease mechanisms and for evaluating potential therapeutic interventions.

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A Mouse Model for Batten Disease


