ELSEVIER

Neurobiology of Disease

www.elsevier.com/locate/ynbdi
Neurobiology of Disease 29 (2008) 242–253

Phenotypic characterization of a mouse model of juvenile neuronal ceroid lipofuscinosis

Martin L. Katz,[a,b,*] Gary S. Johnson,[b] Gregory E. Tullis,[c,1] and Bo Lei[a,d]

[a]*Mason Eye Institute, University of Missouri, Columbia, MO 65212, USA*
[b]*Department of Veterinary Pathobiology, University of Missouri, Columbia, MO 65211, USA*
[c]*Department of Molecular Microbiology and Immunology, University of Missouri, Columbia, MO 65211, USA*
[d]*Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO 65211, USA*

Received 8 June 2007; revised 7 August 2007; accepted 24 August 2007
Available online 7 September 2007

Juvenile neuronal ceroid lipofuscinosis (JNCL) is an autosomal recessively inherited neurodegenerative disorder that results from mutations in the *CLN3* gene. JNCL is characterized by accumulation of autofluorescent lysosomal storage bodies, vision loss, seizures, progressive cognitive and motor decline, and premature death. Studies were undertaken to characterize the neuronal ceroid lipofuscinosis phenotype in a *Cln3* knockout mouse model. Progressive accumulation of autofluorescent storage material was observed in brain and retina of affected mice. The $Cln3^{-/-}$ mice exhibited progressively impaired inner retinal function, altered pupillary light reflexes, losses of inner retinal neurons, and reduced brain mass. Behavioral changes included reduced spontaneous activity levels and impaired learning and memory. In addition, $Cln3^{-/-}$ mice had significantly shortened life spans. These phenotypic features indicate that the mouse model will be useful for investigating the mechanisms underlying the disease pathology in JNCL and provide quantitative markers of disease pathology that can be used for evaluating the efficacies of therapeutic interventions.

Keywords: Behavior; Neurodegeneration; Mouse model; Lysosomal storage; Retina; Brain; Batten disease; Electroretinogram; *CLN3*

Introduction

The neuronal ceroid lipofuscinoses (NCLs) are autosomal recessively inherited lysosomal storage disorders associated with progressive neurodegeneration and the accumulation of autofluorescent storage bodies in various tissues (Wisniewski and Zhong, 2001). In humans, there are a number of forms of NCL that differ from one another in the age at which symptoms first appear, the rate of disease progression, and the patterns of symptoms (Dyken, 1988; Goebel and Wisniewski, 2004; Wisniewski and Zhong, 2001; Wisniewski et al., 2001). Almost all NCL patients exhibit severe vision loss and progressive cognitive impairment that reflects progressive pathological changes in the central nervous system, including engorgement of neurons with storage material, brain and retinal atrophy, and generalized neuronal degeneration (Goebel and Wisniewski, 2004). Currently there are no known treatments to delay or halt the progression of the NCLs; the development of therapeutic interventions has been impeded by the lack of defined objective markers of disease status. In humans, the different forms of NCL result from mutations in at least eight distinct genes (Goebel and Wisniewski, 2004; Siintola et al., 2006, 2007; Steinfeld et al., 2006; Wisniewski et al., 2001).

The most prevalent form of human NCL is the juvenile type, commonly designated Batten disease. Children with this disorder typically begin to exhibit symptoms at 5 to 7 years of age and seldom live past their mid-twenties. Juvenile NCL results from mutations in the *CLN3* gene which encodes a putative membrane protein of unknown function (Consortium, 1995; Kaczmarski et al., 1999; Mao et al., 2003; Mitchison et al., 1997; Phillips et al., 2005; Rakheja et al., 2004). Animal models could be of great value in developing a better understanding of the mechanisms underlying neuropathology in JNCL and in developing therapies for this disorder. Three mouse *Cln3* knockout models have been created (Cotman et al., 2002; Katz et al., 1999; Mitchison et al., 1999) that could be useful in developing therapeutic interventions if they exhibit quantifiable signs of disease. Experiments were performed to determine whether a homozygous *Cln3* knockout mutation in mice resulted phenotypic alterations that resembled the human disease phenotype. This is the first description of a $Cln3^{-/-}$ model that has been studied across the entire life spans of mice and in a mouse strain in which the phenotypic effects of mutations in other NCL genes have also been characterized.

* Corresponding author. University of Missouri School of Medicine, Mason Eye Institute, One Hospital Drive, Columbia, MO 65212, USA. Fax: +1 573 884 4100.
E-mail address: katzm@health.missouri.edu (M.L. Katz).
[1] Current affiliation: Boston University, Department of Ophthalmology, Boston, MA, USA.
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2007.08.017

Materials and methods

Experimental animals

The *Cln3*$^{-/-}$ mouse model for juvenile NCL was generated by gene targeting in RW4 mouse embryonic stem cells as previously described (Katz et al., 1999), and the knockout mice were subsequently backcrossed to the C57BL/6J strain for more than 15 generations. To confirm that the *Cln3*$^{-/-}$ mice were congenic to normal C57BL/6J animals over the entire genome except the region immediately surrounding the *Cln3* gene, the founder mice were genotyped at 110 microsatellite loci that are polymorphic between the C57BL/6J and 129Sv/J (RW4) strains and that span the mouse genome at approximately 15 cM intervals (MaxBax panel, Charles River). Mice that were found to be homozygous for the C57BL/6J alleles at all loci tested were used for further breeding. To ensure that only a small region of the genome surrounding the *Cln3* locus came from the 129Sv/J strain, the mice obtained were also genotyped at polymorphic marker loci D7MIT253, D7MIT105, and D7MIT109 located −7.6 cM, +3.1 cM, and +5.6 cM respectively from the *Cln3* locus (located at 60.4 cM on mouse chromosome 7). Backcrosses were continued until founder mice homozygous for the C57BL/6J genotypes at all three loci were obtained. The resultant mouse model used in these studies is available from the Mutant Mouse Regional Resource Centers (strain 016150; strain name *B6.129X1*-Cln3^{tm1Mkat}; http://www.mmrrc.org/index.html) coordinated by The Jackson Laboratory, Bar Harbor, Maine. C57BL/6J mice that were homozygous for the wild-type *Cln3* allele were used as controls.

Mice were maintained on a 12-h/12-h light/dark cycle and provided with food and water ad libitum. Light intensities at the cage bottoms during the light phase of the daily cycle was 15–25 lx, room temperature was maintained between 18 and 22 °C, and relative humidity was maintained between 30% and 50%. All animal experiments were approved by the University of Missouri–Columbia Animal Care and Use Committee and were conducted in accordance with the Guidelines of the U.S. National Institute of Health regarding the care and use of animals for experimental procedures.

Longevity

Analyses were performed to determine whether the *Cln3* mutation affected longevity. Cohorts of the knockout mice and normal C57BL/6J controls were set aside at weaning and maintained under the standard housing conditions until they died of natural causes. Longevity was determined from records of birth dates and death dates for each animal.

Assessment of storage material accumulation in brain and retina

Mice were euthanized at various ages via carbon dioxide inhalation to determine if the *Cln3*$^{-/-}$ mice exhibited progressive accumulation of autofluorescent lysosomal storage material characteristic of the NCLs. The brains and retinas were immediately removed, fixed, and processed for fluorescence microscopy as described previously (Katz et al., 2005). Sections of frozen tissue were cut at a thickness of 5 μm on a cryostat, mounted in 0.17 M sodium cacodylate buffer, and examined with a Zeiss Axiophot microscope equipped with epi-illumination. Fluorescence emissions were stimulated with light from a 50-W, high-pressure mercury vapor source. Samples were examined and photographed using a 40× Plan Neofluor objective lens with a 1.30 numerical aperture, a 395–440 nm bandpass exciter filter, an FT-460 chromatic beam splitter, and an LP-515 barrier filter. Daylight-balanced color slide film (Koday Elite Chrome 100) was used for photography.

Electron microscopic analyses were also performed to assess the ultrastructure of the storage bodies in the knockout mice. Mice were euthanized with carbon dioxide inhalation at 12 months of age. The brains and eyes were immediately removed and placed in a mixed aldehyde fixative (Katz et al., 2005). With the brain immersed in fixative, coronal brain slices were made centered half way between the front and back edges of the cerebrum and through the center of the cerebellum. The brain slices were incubated in the fixative for at least 2 h at room temperature with gentle agitation. Small regions of the cerebral cortex and cerebellar cortex were then dissected and processed for electron microscopy (Katz et al., 1982). Retina samples were prepared for electron microscopy as described previously (Lei et al., 2006). Thin sections of the tissues were examined and photographed with a JEOL 1200 EX transmission electron microscope.

Spontaneous activity assessment

The spontaneous activities of 12-month-old mutant and control mice were assessed with a VersaMax activity monitoring system (Accuscan Instruments, Inc.). Mice were placed in a plastic enclosure (41 cm × 41 cm × 30.5 cm high) with clear sides and top. The enclosure was transected by an *x*, *y*, *z* array of infrared beams, each with a corresponding detector. The movement and location of the mouse within the enclosure is monitored by recording which light beams are being interrupted by the mouse as a function of time.

Behavioral assessments were performed in an isolated closed room that was maintained under identical conditions for all of the evaluations. The only light in the testing room came from overhead fixtures that produced a mean illumination level in the testing enclosure of 40 lx.

On the day of a test the mice to be evaluated were placed in individual cages in the testing room by 3 h after the onset of the daily light cycle. All testing was performed between 6 and 10 h after light onset. Cages containing mice awaiting testing were placed out of sight of the testing apparatus. To begin testing, a mouse was placed in the monitoring enclosure and electronic monitoring was initiated. During monitoring, the data acquisition computer was covered with an opaque black cloth so that no light from the computer was visible. As soon as testing was initiated, the tester left the room through a revolving darkroom door and did not return to the room until after the end of the test session. Activity of each mouse was monitored for 20 min, however the first 5 min of each session was not included in the analyses to avoid including variability in the time it took the tester to cover the computer and leave the room. At the end of each monitoring session, the test enclosure was cleaned to remove any residue left by the mouse. The spontaneous activities of six normal C57BL/6J and seven *Cln3*$^{-/-}$ mice were compared.

Learning and memory assessment

Learning and memory were assessed in the *Cln3*$^{-/-}$ and control mice using the VersaMax activity monitoring system equipped with a hole poke accessory. This accessory consisted of a floor with 16 equally spaced holes. Beneath each hole was a well into which a piece of food bait (a piece of peanut butter flavored cereal) was