Finding the most appropriate mouse model of juvenile CLN3 (Batten) disease for therapeutic studies: the importance of genetic background and gender

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ABSTRACT
Mutations in the CLN3 gene cause a fatal neurodegenerative disorder: juvenile CLN3 disease, also known as juvenile Batten disease. The two most commonly utilized mouse models of juvenile CLN3 disease are Cln3-knockout (Cln3−/−) and Cln3Δex7/8-knock-in mice, the latter mimicking the most frequent disease-causing human mutation. To determine which mouse model has the most pronounced neurological phenotypes that can be used as outcome measures for therapeutic studies, we compared the exploratory activity, motor function and depressive-like behavior of 1-, 3- and 6-month-old Cln3−/− and Cln3Δex7/8-knock-in mice on two different genetic backgrounds (129S6/SvEv and C57BL/6J). Although, in many cases, the behavior of Cln3−/− and Cln3Δex7/8 mice was similar, we found genetic-background-, gender- and age-dependent differences between the two mouse models. We also observed large differences in the behavior of the 129S6/SvEv and C57BL/6J wild-type strains, which highlights the strong influence that genetic background can have on phenotype. Based on our results, Cln3−/− male mice on the 129S6/SvEv genetic background are the most appropriate candidates for therapeutic studies. They exhibit motor deficits at 1 and 6 months of age in the vertical pole test, and they were the only mice to show impaired motor coordination in the rotarod test at both 3 and 6 months. Cln3−/− males on the C57BL/6J background and Cln3Δex7/8 males on the 129S6/SvEv background also provide good outcome measures for therapeutic interventions. Cln3−/− (C57BL/6J) males had serious difficulties in climbing down (at 1 and 6 months) and turning downward on (at 1, 3 and 6 months) the vertical pole, whereas Cln3Δex7/8 (129S6/SvEv) males climbed down the vertical pole drastically slower than wild-type males at 3 and 6 months of age. Our study demonstrates the importance of testing mouse models on different genetic backgrounds and comparing males and females in order to find the most appropriate disease model for therapeutic studies.

KEY WORDS: Juvenile neuronal ceroid lipofuscinosis, Batten disease, CLN3, Cln3−/− mouse model, Cln3Δex7/8-knock-in mouse model, 129S6/SvEv, C57BL/6J

INTRODUCTION
Neuronal ceroid lipofuscinoses, also known as Batten disease, are a group of recessively inherited, fatal lysosomal storage disorders characterized by progressive neurodegeneration and the intracellular accumulation of autofluorescent lipopigment (Goebel and Wisniewski, 2004). Batten disease is a rare disease and mostly affects children. Based primarily on the age of onset, Batten disease is classified into six clinical forms: congenital, infantile, late infantile, juvenile, Northern epilepsy and adult (Kufs disease) (Schulz et al., 2013). Vision loss, seizures, and progressive cognitive and motor decline are the common symptoms, and the neurodegeneration ultimately leads to early death. The different forms of Batten disease are currently associated with mutations in 14 genes (Warrier et al., 2013).

Mutations of the CLN3 gene cause the majority of the prevalent, juvenile-onset, form of Batten disease, and this disorder is now called juvenile CLN3 disease to clearly identify the genetic cause and clinical form (International Batten Disease Consortium, 1995; Williams and Mole, 2012). The disease begins between 4 and 10 years of age, and reaches its terminal stage in the late teens or early 20s. CLN3 encodes a putative lysosomal/endosomal transmembrane protein, but the exact function of CLN3 and why CLN3 mutations cause selective neurodegeneration are still unknown. No specific treatment is currently available that could halt or slow the progression of the disease.

The two most commonly utilized mouse models of juvenile CLN3 disease are Cln3-knockout (Cln3−/−) and Cln3Δex7/8-knock-in mice. The Cln3Δex7/8 model was generated by targeted recombination to remove exons 7 and 8 from the endogenous Cln3 gene. This created a ‘knock-in’ of exon 7/8-deleted Cln3, to mimic the most frequent disease-causing human mutation (Catman et al., 2002). The Cln3−/− model was created by replacing the start codon and the first 12 amino acids of the Cln3 gene. This created a ‘knock-out’ of the Cln3 gene. Both Cln3Δex7/8 and Cln3−/− mice on the 129S6/SvEv genetic background have been extensively studied (Chan et al., 2009; Kovács and Pearce, 2008; Kovács et al., 2012; Kovács et al., 2011; Kovács et al., 2006; Osório et al., 2009; Pears et al., 2005; Pontikis et al., 2004; Sehner et al., 2011; Seigel et al., 2002; Weimer et al., 2007; Weimer et al., 2009; Weimer et al., 2006). The Cln3Δex7/8-knock-in mouse model was generated and initially characterized on a mixed 129S6/SvEv × CD1 genetic background (Catman et al., 2002; Pontikis et al., 2005). In subsequent studies, Cln3Δex7/8 mice inbred either on the C57BL/6J or C57BL/6N genetic background were used (Finn et al., 2011; Osório et al., 2009; Staropoli et al., 2012). Both Cln3Δex7/8 and Cln3−/− mice showed characteristic features of the human disease, including intracellular accumulation of autofluorescent storage material (Catman et al., 2002; Mitchison et al., 1999), astrocytosis and microglial activation (Catman et al., 2002; Pontikis et al., 2004; Pontikis et al., 2005; Weimer et al., 2009), neuronal loss (Pontikis et al., 2005), and vision loss (Weimer et al., 2007).
TRANSLATIONAL IMPACT

Clinical issue
Batten disease, also known as neuronal ceroid lipofuscinoses, is a group of rare fatal lysosomal storage disorders characterized by progressive neuronal degeneration. The disease mostly affects children. Mutations of the CLN3 gene cause the majority of the most prevalent, juvenile-onset, form of the disease, which presents between 4 and 10 years of age and reaches its terminal stage in the late teens or early 20s. No specific treatment is currently available to halt or slow disease progression. To study the pathomechanisms of juvenile Batten disease and develop therapeutic approaches, various mouse models have been developed. It has not been determined, however, which mouse model on which genetic background is the most suitable for therapeutic studies.

Results
The two most commonly utilized mouse models of juvenile Batten disease are Cln3-knockout (Cln3−/−) and Cln3Δex7/8-knock-in mice, the latter mimicking the most frequent disease-causing human mutation. To determine which mouse model on which genetic background has the most pronounced neurological phenotypes that can be used as outcome measures for therapeutic studies, the authors compared the exploratory activity, motor function and depressive-like behavior of 1-, 3- and 6-month-old Cln3−/− and Cln3Δex7/8-knock-in mice on two different genetic backgrounds (129S6/SvEv and C57BL/6J). The authors found genetic-background-, gender- and age-dependent differences between the two mouse models. Large differences in the behavior of the 129S6/SvEv and C57BL/6J wild-type strains were also observed, highlighting the strong influence that genetic background can have on phenotype. Based on the authors’ results, Cln3−/− male mice on the 129S6/SvEv genetic background are the most appropriate candidates for therapeutic studies. They exhibited motor deficits at 1 and 6 months of age in the vertical pole test, and were the only mice to show impaired motor coordination in the rotated test at both 3 and 6 months.

Implications and future directions
This study provides the first behavioral comparison of Cln3−/− and Cln3Δex7/8-knock-in mice on identical genetic backgrounds. The differences observed reveal that, unlike as previously thought, Cln3Δex7/8 mice might not be true nulls but express residual truncated CLN3. The study demonstrates the importance of testing mouse models on different genetic backgrounds and comparing males and females in order to find the most appropriate disease model for therapeutic studies. Using the most suitable mouse model of a human disease in preclinical drug testing greatly enhances the chance of advancing to successful clinical trials.

RESULTS

Cln3−/− and Cln3Δex7/8-knock-in mice have genotype-background-, gender- and age-dependent differences in exploratory activity

The exploratory behavior of mice was assessed in the dish test, measuring the time that a mouse stayed in a large Petri dish. It should be noted, however, that activity level and anxiety also affect the outcome of the dish test. In this test, we found a large difference between the two wild-type (WT) strains: whereas most 129S6/SvEv mice, both males and females, stayed in the Petri dish for 6 minutes (the time limit of the test), the majority of C57BL/6J mice left the dish in seconds (Fig. 1, asterisks indicate the statistically significant differences between the two WT strains, comparing males to males, and females to females). Because of this extreme difference, results on the two genetic backgrounds are described separately.

Results on the 129S6/SvEv background
One-month-old Cln3−/− mice, both males and females, spent considerably less time in a large Petri dish than Cln3Δex7/8 or WT mice (Fig. 1, top graph). At the age of 3 or 6 months, however, the exploratory activity of WT, Cln3−/− and Cln3Δex7/8 mice was similar (Fig. 1, middle and bottom graphs).

Results on the C57BL/6J background
At both 1 and 3 months of age there were no differences in the exploratory behavior of Cln3−/− and Cln3Δex7/8 mice (Fig. 1, top and middle graph). Three-month-old Cln3Δex7/8 females, however, showed decreased exploratory activity as compared to C57BL/6J WT females (Fig. 1, middle graph). Similarly, the exploratory activity of 6-month-old Cln3Δex7/8 females was decreased compared to WT, and this time to Cln3−/− females as well (Fig. 1, bottom graph). At the same time, 6-month-old Cln3−/− males showed enhanced exploratory activity in comparison to C57BL/6J WT males (Fig. 1, bottom graph). The difference between 6-month-old Cln3−/− and Cln3Δex7/8 males did not reach statistical significance (Fig. 1, bottom graph).

Genetic-background-, gender- and age-dependent differences in the motor phenotypes of Cln3−/− and Cln3Δex7/8-knock-in mice

After the dish test, the same mice were also tested in a modified vertical pole test. This test measures the balance, spatial orientation and motor coordination, although anxiety or motivation to move can also affect the test results.

Climbing down the vertical pole
First, the ability of mice to climb down a vertical pole was examined (Fig. 2). Whereas WT mice climbed down the pole quickly and touched the base of the pole without hesitation, Cln3−/− and Cln3Δex7/8 mice showed abnormal behavior, which included turning upward, falling off the pole, slowly descending, freezing on the pole and hesitating to touch the base of the pole. Cln3−/− males on both genetic backgrounds climbed down significantly slower than their WT counterparts at 1 and 6 months of age. Whereas Cln3Δex7/8 males on the C57BL/6J background showed the same age-dependent
motor deficit as $Cln3^{-/-}$ males (at 1 and 6 months), $Cln3^{Δex7/8}$ males on the 129S6/SvEv background exhibited a marked delay in climbing down at 3 and 6 months as compared to WT males (Fig. 2). The differences between $Cln3^{-/-}$ and $Cln3^{Δex7/8}$ males on either genetic background did not reach statistical significance at 1, 3 or 6 months. The climbing-down ability of $Cln3^{-/-}$ and $Cln3^{Δex7/8}$ females, however, varied depending on age and genetic background. On the C57BL/6J background, 1- and 3-month-old $Cln3^{-/-}$ females climbed down the pole dramatically slower than $Cln3^{Δex7/8}$ or WT females (Fig. 2, top and middle graphs). At 6 months, however, $Cln3^{-/-}$ and $Cln3^{Δex7/8}$ females (on C57BL/6J) showed similar motor deficits as compared to WT females (Fig. 2, bottom graph).

Turning downward on the vertical pole

The ability of mice to turn downward on a vertical pole was also tested. There was a huge difference between 129S6/SvEv and C57BL/6J WT mice: the majority of 129S6/SvEv mice (both males and females) did not turn downward at all during the test trials (Fig. 3). On the 129S6/SvEv background, only 1-month-old $Cln3^{-/-}$
and Cln3<sup>Δex7/8</sup> females exhibited a phenotype: a statistically significant delay in turning downward as compared to WT females (Fig. 3, top graph).

On the C57BL/6J background, Cln3<sup>−/−</sup> males at all three ages turned downward substantially later than WT males, whereas Cln3<sup>Δex7/8</sup> males were only different from WT males at 1 month of age (Fig. 3). The turning-downward performance of 1-month-old Cln3<sup>−/−</sup> males, however, was significantly worse than that of 1-month-old Cln3<sup>−/−</sup> males (Fig. 3, top graph). Cln3<sup>−/−</sup> females on the C57BL/6J background had an impaired ability to turn downward at 3 months of age as compared to their WT or Cln3<sup>Δex7/8</sup> counterparts (Fig. 3, middle graph). In contrast, at 6 months, Cln3<sup>Δex7/8</sup> females (on C57BL/6J) showed a considerable delay in turning downward, whereas Cln3<sup>−/−</sup> females (on C57BL/6J) performed like WTs (Fig. 3, bottom graph).

**Rotarod test**

The rotarod test assesses balance and motor coordination, although motor learning capability and endurance level can also affect the rotarod performance. The sensitivity of the rotarod test depends on the task parameters, particularly on the acceleration (Carter et al., 1999; Pallier et al., 2009; Rustay et al., 2003). After trying different...
rotarod protocols and accelerations in a previous study (Kovács and Pearce, 2013), we found that a 0.2 rpm/s acceleration is the most suitable to detect even slight differences in rotarod performance. At 3 and 6 months of age, 1 day after the dish test, vertical pole test and tail suspension test, the same mice were also tested in an accelerating rotarod test (0.2 rpm/s starting from 0 rpm). At both times, only Cln3Δex7/8 males on the 129S6/SvEv background showed impaired rotarod performance (Fig. 4). Our findings that 3-month-old Cln3Δex7/8 females, 3- and 6-month-old Cln3Δex7/8 (129S6/SvEv) mice on two different genetic backgrounds (129S6/SvEv and C57BL/6J), as well as 129S6/SvEv and C57BL/6J WT mice, were tested in an accelerating rotarod test (0.2 rpm/s starting from 0 rpm). Cln3Δex7/8, but not Cln3Δex7/8 knock-in, males on the 129S6/SvEv background fell from the rotating rod significantly sooner than WT males. Columns and bars represent means ± s.e.m. (n=10-12). The same mice were tested at 3 and 6 months of age. Statistical significance was determined by 1-way ANOVA with Bonferroni’s post-test for multiple comparisons. Asterisks indicate significant differences between 129S6/SvEv and C57BL/6J WT mice, males versus males, and females versus females: *P<0.05, **P<0.01 and ****P<0.0001.

extraordinary rotarod performance of Cln3Δex7/8 females is that the Cln3Δex7/8-derived truncated CLN3 increased the motivation of staying on the rotating rod and/or the endurance specifically in females.

We also compared the rotarod performance of the two WT strains and found that 3-month-old 129S6/SvEv males and females, and 6-month-old 129S6/SvEv females, fell from the rotating rod significantly sooner than their C57BL/6J counterparts (Fig. 4).

Genetic-background-, gender- and age-specific differences in the depressive-like behavior of Cln3Δex7/8- and Cln3Δex7/8-derived truncated CLN3 increased the motivation of staying on the rotating rod and/or the endurance specifically in females.

The same mice in the vertical pole test, however, showed either motor deficits [3- and 6-month-old Cln3Δex7/8 (129S6/SvEv) females, 6-month-old Cln3Δex7/8 (C57BL/6J) females] or were not different from WTs [3-month-old Cln3Δex7/8 (C57BL/6J) females] (see Figs 2, 3). Based on the above, a possible explanation for the extraordinary rotarod performance of Cln3Δex7/8 females is that the Cln3Δex7/8-derived truncated CLN3 increased the motivation of staying on the rotating rod and/or the endurance specifically in females.

At 6 months of age, Cln3Δex7/8 (129S6/SvEv) males spent significantly less time immobilized than Cln3Δex7/8 (129S6/SvEv) or WT males (Fig. 5, bottom graph). Furthermore, 6-month-old Cln3Δex7/8 (129S6/SvEv) females, Cln3Δex7/8 (C57BL/6J) males and especially Cln3Δex7/8 (C57BL/6J) males exhibited abnormally increased desperation and stayed immobilized for a shorter time than their WT counterparts (Fig. 5, bottom graph; Fig. 6C). The time course plot revealed a statistically significant difference between Cln3Δex7/8 (C57BL/6J) and Cln3Δex7/8 (C57BL/6J) males in the 5th minute (Fig. 6C).

We also compared the depressive-like behavior of the two WT strains and found that 1- and 3-month-old C57BL/6J males and females, and 6-month-old C57BL/6J males stayed immobilized substantially longer than their 129S6/SvEv counterparts (Fig. 5; Fig. 6D-F).

All the other time courses of immobility not presented in Fig. 6 are shown in supplementary material Figs S1-S3.

Genetic-background-, gender- and age-specific differences in the weight of Cln3Δex7/8 and Cln3Δex7/8-derived truncated CLN3 increased the motivation of staying on the rotating rod and/or the endurance specifically in females.

For weight comparison, mice were weighed 30-40 minutes before starting the behavioral tests with them. The same mice were weighed at 1, 3 and 6 months of age. On the C57BL/6J genetic background, no weight differences were found between Cln3Δex7/8 and Cln3Δex7/8 mice or between WT and mutant mice at any age (Fig. 7). On the 129S6/SvEv background, 1-month-old Cln3Δex7/8 mice, both males and females, were strikingly heavier than Cln3Δex7/8 mice (Fig. 7, top graph). One-month-old Cln3Δex7/8 (129S6/SvEv) females were also markedly heavier than WT 129S6/SvEv females. At the same time, 1-month-old Cln3Δex7/8 (129S6/SvEv) females were significantly lighter than 129S6/SvEv females (Fig. 7, top graph). At 3 months of age, Cln3Δex7/8 (129S6/SvEv) females were considerably heavier than Cln3Δex7/8 (129S6/SvEv) females (Fig. 7, middle graph). Because the observed weight differences might affect the performance in behavioral tests, particularly in motor skill tests, we carried out correlation analyses between the weight and the
behavioral test results. The weight of 1- and 3-month-old 129S6/SvEv, Cln3Δex7/8 (129S6/SvEv) and Cln3 Δex7/8 (129S6/SvEv) mice did not correlate with their performance in any of the behavioral tests, indicating that, in our study, the weight differences did not influence the behavioral test results.

We also compared the weights in the two WT strains and found that 129S6/SvEv males were significantly lighter than C57BL/6J males at 3 and 6 months (Fig. 2). It should be noted that various other behavioral tests (e.g. cognitive tests, open field, light-dark box, gait analysis, variations of rotares) not used in our study could provide additional valuable readouts for one or more of the models. Similarly, other genetic backgrounds might prove superior in resolving CLN3-deficient behavioral phenotypes.

Our current selection of the most appropriate mouse models was based solely on behavioral tests. Because mouse models of juvenile CLN3 disease show age-dependent neuropathological changes, including microglial activation, astrogliosis and localized neuronal loss (Cotman et al., 2002; Pontikis et al., 2004; Pontikis et al., 2005; Weimer et al., 2009), brain pathology is another important (but terminal) outcome measure for therapeutic studies. Further studies will determine whether the neurological deficits in our chosen models correlate with the severity and progression of neuropathological changes.

**Similarities and differences in the neurological phenotypes of Cln3Δ−/− and Cln3Δex7/8−/− knock-in mice**

The mouse Cln3Δex7/8 gene mimics the most frequent disease-causing human mutation (Cotman et al., 2002). The mutant Cln3Δex7/8 (mouse) and CLN3Δex7/8 (human) genes theoretically produce a truncated protein, which might have a residual function (Kitzmüller et al., 2008). Evidence suggests, however, that cellular quality-control mechanisms at the RNA and protein levels degrade the mutant human and mouse transcript and polypeptide (Chan et al., 2008), and, thus, both CLN3Δex7/8 (human) and Cln3Δex7/8 (mouse) are likely null mutations. If Cln3Δex7/8 is indeed a null mutation then the behavioral phenotypes and their progression in the Cln3Δ−/− and Cln3Δex7/8−/− knock-in mouse models of juvenile Batten disease should be very similar. Our results show that, although in many cases the behavior of Cln3Δ−/− and Cln3Δex7/8−/− mice is similar, genetic-background-, gender- and age-dependent differences existed between the two mouse models, Cln3Δ−/− mice showing more severe phenotypes in some cases whereas Cln3Δex7/8−/− mice exhibited more pronounced behavioral deficits in other instances. This could suggest that, in Cln3Δex7/8−/− mice, a truncated Cln3 transcript has enough expression to impart a residual but presumably partially functional Cln3 gene product. In a few instances, however, the behavioral defect was more severe or only exhibited in Cln3Δex7/8−/− mice, indicating a detrimental effect of truncated CLN3 in

**Fig. 5. Genetic-background-, gender- and age-specific differences in the depressive-like behavior of Cln3−/− and Cln3Δex7/8−/− knock-in mice.** The depressive-like behavior of 1-, 3- and 6-month-old Cln3−/− and Cln3Δex7/8−/− knock-in (Cln3KI) mice on two different genetic backgrounds (129S6/SvEv and C57BL/6J) was assessed by the tail suspension test. 129S6/SvEv and C57BL/6J WT mice served as controls. Mice were suspended 45 cm above table level, and the duration of immobility was measured in 1-minute bins for 6 minutes. The graphs show the total time of immobility in the 6 minutes of the test; columns and bars represent mean±s.e.m. (n=10-12). The same mice were tested at 1, 3 and 6 months of age. Statistical significance was determined by 1-way ANOVA with Bonferroni’s post-test for multiple comparisons. Asterisks indicate significant differences between 129S6/SvEv and C57BL/6J WT mice, males versus males, and females versus females: *P<0.05 and ****P<0.0001.

**DISCUSSION**

**Mouse models of juvenile CLN3 disease: suitability for therapeutic studies**

In the present study, we compared the exploratory activity, motor function and depressive-like behavior of Cln3−/− and Cln3Δex7/8−/− knock-in mice on two different genetic backgrounds (129S6/SvEv and C57BL/6J) to determine which mouse model on which genetic background has the most pronounced neurological phenotypes that can be used as outcome measures for therapeutic studies. Based on our results, Cln3−/− male mice on the 129S6/SvEv genetic background are the most appropriate candidates for therapeutic studies. They show motor deficits at 1 and 6 months of age in the vertical pole test (Fig. 2), and they were the only mice exhibiting impaired motor coordination in the rotares test at both 3 and 6 months (Fig. 4). Cln3Δ−/− males on the C57BL/6J background and Cln3Δex7/8−/− males on the 129S6/SvEv genetic background also provide good outcome measures for therapeutic interventions. Cln3Δ−/− (C57BL/6J) males had serious difficulties in climbing down (at 1 and 6 months) and turning downward on (at 1, 3 and 6 months) the vertical pole (Figs 2-3), whereas Cln3Δex7/8 (129S6/SvEv) males climbed down the vertical pole drastically slower than WT males at 3 and 6 months of age (Fig. 2). It should be noted that various other behavioral tests (e.g. cognitive tests, open field, light-dark box, gait analysis, variations of rotares) not used in our study could provide additional valuable readouts for one or more of the models. Similarly, other genetic backgrounds might prove superior in resolving CLN3-deficient behavioral phenotypes.

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comparison to the complete lack of CLN3 in Cln3<sup>−/−</sup> mice. The slight differences in the genetic background between our Cln3<sup>−/−</sup> and Cln3<sub>Δex7/8</sub> mice (see Materials and Methods) might also contribute to the observed behavioral differences.

Recent studies in the Cln3<sup>−/−</sup> and Cln3<sub>Δex7/8</sub> mouse models indicate that juvenile Batten disease has a neurodevelopmental component resulting in early disease phenotypes (at ≤1 month of age) in these mice (Osório et al., 2009; Weimer et al., 2009). Some of these early neurological problems, however, might be reduced or eliminated by compensatory mechanisms during later brain development and maturation [see e.g. Cln3<sup>−/−</sup> (129S6/SvEv) males and females in the dish test (Fig. 1); Cln3<sup>−/−</sup> (129S6/SvEv) females, Cln3<sup>−/−</sup> (C57BL/6J) males and Cln3<sub>Δex7/8</sub> (C57BL/6J) males in the climb-down test (Fig. 2); Cln3<sup>−/−</sup> (129S6/SvEv) females, Cln3<sub>Δex7/8</sub> (129S6/SvEv) females and Cln3<sub>Δex7/8</sub> (C57BL/6J) males in the turn-downward test (Fig. 3); and Cln3<sup>−/−</sup> (129S6/SvEv) females, Cln3<sub>Δex7/8</sub> (129S6/SvEv) females and Cln3<sub>Δex7/8</sub> (C57BL/6J) males in the turn-downward test (Fig. 3); and Cln3<sub>Δex7/8</sub> (C57BL/6J) females in the tail suspension test (Fig. 5)]. Nevertheless, progressive neurological deficits, depending on the genetic background and the disease model, are also evident [see e.g. Cln3<sub>Δex7/8</sub> (129S6/SvEv) males in the climb-down test (Fig. 2); Cln3<sup>−/−</sup> (129S6/SvEv) females, Cln3<sub>Δex7/8</sub> (129S6/SvEv) females and Cln3<sub>Δex7/8</sub> (C57BL/6J) males in the turn-downward test (Fig. 3); and Cln3<sup>−/−</sup> (129S6/SvEv) females in the tail suspension test (Fig. 5)].

Our results also indicate that the vertical pole test is the most representative to detect the motor phenotype of juvenile Batten disease. This was the only test that showed deficits for both Cln3<sup>−/−</sup>
phenotype for the C57BL/6J background. Downward and climb-down time would reveal a stronger motor function in C57BL/6J mice on the 129S6/SvEv genetic background than on C57BL/6J WT mice (Figs 1, 3-5). Our findings in the rotarod test, tail suspension test (dramatically longer immobility of C57BL/6J than 129S6/SvEv mice) are in agreement with previous reports (Cook et al., 2002; Paulus et al., 1999; Van Bogaert et al., 2006). The large behavioral differences between the two WT strains highlight the strong influence that genetic background can have on the disease phenotypes of transgenic mice. In our study, for example, Cln3Δex7/8-knock-in mice only on the 129S6/SvEv background had motor deficits at 3 months of age (Fig. 2). The genetic background, as several studies have shown, can modify, and even suppress, the effect of a transgene or gene deletion (Bilovocky et al., 2003; Duyser and Lockridge, 2006; Kelly et al., 1998; Lariviere et al., 2001; Lloret et al., 2006; Magara et al., 1999; Mahajan et al., 2004; Nguyen et al., 1997; Tang et al., 2003; Yang et al., 2005).

Although C57BL/6J embryonic stem cell lines became available in recent years, most transgenic mice had been generated using 129S6/SvEv embryonic stem cells. Because 129S6/SvEv mice have a neuroanatomical defect (hypoplasia of the corpus callosum in 60-80% of the mice) (Balogh et al., 1999), carry the Disrupted-in-Schizophrenia 1 (DISC1) mutation (Kim et al., 2012; Koike et al., 2006), and do not perform well in learning tests (Balogh et al., 1999), a common practice has been to backcross 129S6/SvEv transgenic mice to the C57BL/6J genetic background. The C57BL/6J background, however, also has disadvantages. As compared to 129S6/SvEv mice, C57BL/6J mice are hyperactive and aggressive, probably partly due to the fact that the cell surface expression of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate)- and NMDA (N-methyl-D-aspartate)-type glutamate receptors are markedly higher in the brain of C57BL/6J mice (Finn et al., 2010). Furthermore, C57BL/6J mice have a relatively low bone density (Sheng et al., 1999), develop age-related hearing loss (Johnson et al., 1997), and are also susceptible to diet-induced obesity (Collins et al., 2004), type 2 diabetes (Freeman et al., 2006; Parekh et al., 1998) and atherosclerosis (Nishina et al., 1990; Paigen et al., 1985). Because glutamate receptor function is already highly enhanced in C57BL/6J neurons as compared to 129S6/SvEv mice (Finn et al., 2010), the 129S6/SvEv background seems to be more appropriate for studying neurodegenerative diseases where enhanced glutamate receptor function might be involved in the pathophysiology.

The variable neuroanatomical defect of hypoplastic corpus callosum in 60-80% of 129S6/SvEv mice (Balogh et al., 1999) could have a significant effect in behavioral tests. If this is the case, variation in the test results should be higher on the 129S6/SvEv background (intact corpus callosum in 20-40% of the mice) than on the C57BL/6J background (intact corpus callosum in all mice). In our vertical pole test, rotarod test and tail suspension test, however, as the error bars in Figs 2-5 show, the test result variations on the two genetic backgrounds were very similar.

In summary, our study demonstrates the importance of testing mouse models on different genetic backgrounds and comparing males and females in order to find the most appropriate disease model for therapeutic studies. In addition, we provide the first behavioral comparison of Cln3Δex7/8 and Cln3Δex7/8-knock-in mice on identical genetic backgrounds, revealing that the Cln3Δex7/8 mice might differ from Cln3Δex7/8 mice due to residually expressed truncated CLN3.

Choice of the genetic background for mouse models of neurodegenerative and neurological diseases

We observed large differences in the behavior of the 129S6/SvEv and C57BL/6J WT strains (Figs 1, 3-5). Our findings in the rotarod test (better performance of C57BL/6J than 129S6/SvEv mice) and in the tail suspension test (dramatically longer immobility of C57BL/6J than 129S6/SvEv mice) are in agreement with previous reports (Cook et al., 2002; Kelly et al., 1998; Miller et al., 2010). Substantial differences in the locomotor activity, stress reactivity and anxiety-related behaviors of 129S6/SvEv and C57BL/6J mice have also been demonstrated (Cook et al., 2002; Paulus et al., 1999; Van Bogaert et al., 2006). The large behavioral differences between the two WT strains highlight the strong influence that genetic background can have on the disease phenotypes of transgenic mice. In our study, for example, Cln3Δex7/8-knock-in mice only on the 129S6/SvEv genetic background had motor deficits at 3 months of age (Fig. 2). The genetic background, as several studies have shown, can modify, and even suppress, the effect of a transgene or gene deletion (Bilovocky et al., 2003; Duyser and Lockridge, 2006; Kelly et al., 1998; Lariviere et al., 2001; Lloret et al., 2006; Magara et al., 1999; Mahajan et al., 2004; Nguyen et al., 1997; Tang et al., 2003; Yang et al., 2005).

Although C57BL/6J/embryonic stem cell lines became available in recent years, most transgenic mice had been generated using 129S6/SvEv embryonic stem cells. Because 129S6/SvEv mice have a neuroanatomical defect (hypoplasia of the corpus callosum in 60-80% of the mice) (Balogh et al., 1999), carry the Disrupted-in-Schizophrenia 1 (DISC1) mutation (Kim et al., 2012; Koike et al., 2006), and do not perform well in learning tests (Balogh et al., 1999), a common practice has been to backcross 129S6/SvEv transgenic mice to the C57BL/6J genetic background. The C57BL/6J background, however, also has disadvantages. As compared to 129S6/SvEv mice, C57BL/6J mice are hyperactive and aggressive, probably partly due to the fact that the cell surface expression of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate)- and NMDA (N-methyl-D-aspartate)-type glutamate receptors are markedly higher in the brain of C57BL/6J mice (Finn et al., 2010). Furthermore, C57BL/6J mice have a relatively low bone density (Sheng et al., 1999), develop age-related hearing loss (Johnson et al., 1997), and are also susceptible to diet-induced obesity (Collins et al., 2004), type 2 diabetes (Freeman et al., 2006; Parekh et al., 1998) and atherosclerosis (Nishina et al., 1990; Paigen et al., 1985). Because glutamate receptor function is already highly enhanced in C57BL/6J neurons as compared to 129S6/SvEv mice (Finn et al., 2010), the 129S6/SvEv background seems to be more appropriate for studying neurodegenerative diseases where enhanced glutamate receptor function might be involved in the pathophysiology.

The variable neuroanatomical defect of hypoplastic corpus callosum in 60-80% of 129S6/SvEv mice (Balogh et al., 1999) could have a significant effect in behavioral tests. If this is the case, variation in the test results should be higher on the 129S6/SvEv background (intact corpus callosum in 20-40% of the mice) than on the C57BL/6J background (intact corpus callosum in all mice). In our vertical pole test, rotarod test and tail suspension test, however, as the error bars in Figs 2-5 show, the test result variations on the two genetic backgrounds were very similar.

In summary, our study demonstrates the importance of testing mouse models on different genetic backgrounds and comparing males and females in order to find the most appropriate disease model for therapeutic studies. In addition, we provide the first behavioral comparison of Cln3Δex7/8 and Cln3Δex7/8-knock-in mice on identical genetic backgrounds, revealing that the Cln3Δex7/8 mice might differ from Cln3Δex7/8 mice due to residually expressed truncated CLN3.

MATERIALS AND METHODS

Animals

We maintained Cln3Δex7/8 mice on the 129S6/SvEv genetic background (Cln3Δex7/8 mice were originally backcrossed with 129S6/SvEv mice for 12
were calculated for each mouse. To compare the two mouse models we backcrossed Clin3\textsuperscript{−/−} (129S6/SvEv) mice to the C57BL/6J, and Clin3\textsuperscript{+/+} (C57BL/6J) mice to the 129S6/SvEv genetic background for ten generations. To verify the genetic background of the mouse strains used in our study, tail snips of representative mice (two males and two females from each strain) were sent to The Jackson Laboratory (Bar Harbor, Maine) for genome scanning. Analysis of 158 SNPs polymorphic between the C57BL/6 and 129 strains and covering 19 autosomes and the X chromosome with a density of ~15-20 Mb showed that our Clin3\textsuperscript{−/−} (129S6/SvEv) and Clin3\textsuperscript{+/+} (129S6/SvEv) mice have 99.4% and 97.6% of their genome, respectively, from the 129S6/SvEv strain, and our Clin3\textsuperscript{−/−} (C57BL/6J) and Clin3\textsuperscript{+/+} (C57BL/6J) mice have 99.4% and 98.1% of their genome, respectively, from the C57BL/6J strain (supplementary material Table S1).

129S6/SvEv and C57BL/6J WT mice maintained in our mouse colony were the controls in the behavioral experiments. Mice were housed in individually vented microisolator cages (four or five mice/cage) with ad libitum access to food and water. Mice were fed with the Teklad Global 2918 diet (Harlan Laboratories, Indianapolis, IN), and their drinking water was tap water. All procedures were carried out according to the guidelines of the Animal Welfare Act and NIH policies, and were approved by the Sanford Research Animal Care and Use Committee.

**Behavioral testing**

Mice were transported to the behavioral testing room where the lights had been dimmed. Mice were labeled on their tails for easy identification, weighed, and were allowed to acclimatize to the room for at least 20 minutes before starting the behavioral tests. All mice were tested first in the dish test, then in the modified vertical pole test, and finally in the tail suspension test. One day later (in the case of 3- and 6-month-old mice) the same mice were also tested in the rotarod test. All behavioral tests were carried out under dim light to keep the anxiety level of mice minimal. The same mice were tested at 1, 3 and 6 months of age.

**Dish test**

The dish test measures the exploratory behavior of mice, although activity level and anxiety also affect the outcome of this test. The mouse was placed in a large plastic Petri dish (diameter: 15 cm; height: 1.5 cm) located in the middle of a clean table, and the time until the mouse stepped out of the dish with all four legs was measured in a single trial. The time limit of the trial was 6 minutes. The Petri dish was cleaned between mice from the same cage (same genotype and gender), and a new Petri dish was used for each cage.

**Modified vertical pole test**

With the vertical pole, the balance, spatial orientation and motor coordination of mice are tested. However, anxiety or motivation to move might also affect the test results. In the traditional vertical pole test (see e.g. Iwamoto et al., 2003), the mouse is placed on top of the pole head upward, and the time until it turns downwards and climbs down to the base of the pole is measured. Because the majority of our mice on the 129S6/SvEv genetic background, including WT mice, do not turn downward at all (see Fig. 3), we modified the vertical pole test so it starts with the climb down. The vertical pole was an all-thread metal rod (diameter: 1.27 cm; height: 66 cm), screwed to a 3.81-cm-thick plastic block (24.5 cm×25.4 cm). The plastic block was covered with a 3.81-cm-thick green hunting seat cushion (nitrile rubber/PVC foam) to prevent the mice from injury if they fell from the pole. The height of the pole measured from the surface of the hunting seat cushion (seat cushion was 59 cm). The mouse was placed, head downward, on top of the pole. The height of the vertical pole was measured. Because the majority of our mice on the 129S6/SvEv genetic background, including WT mice, do not turn downward at all (see Fig. 3), we modified the vertical pole test so it starts with the climb down. The vertical pole was an all-thread metal rod (diameter: 1.27 cm; height: 66 cm), screwed to a 3.81-cm-thick plastic block (24.5 cm×25.4 cm). The plastic block was covered with a 3.81-cm-thick green hunting seat cushion (nitrile rubber/PVC foam) to prevent the mice from injury if they fell from the pole. The height of the pole measured from the surface of the hunting seat cushion (seat cushion was 59 cm). The mouse was placed, head downward, on top of the pole. The height of the vertical pole was measured.

**Statistical analysis**

Statistical analysis was performed using GraphPad Prism 5.04 (GraphPad Software, San Diego, CA). Most of the data sets from the vertical pole test, rotarod test, tail suspension test and weight measurement passed the normality test (alpha level 0.05); therefore, one-way ANOVA with Bonferroni’s post-test was used for comparison in these behavioral tests and for weight comparison as well. Because most data sets from the dish test did not pass the normality test (alpha level 0.05), the non-parametric Kruskal-Wallis test with Dunn’s post-test was applied for comparison in this case. To compare the time courses in the tail suspension test, repeated measures 2-way ANOVA with Bonferroni’s post-test was used. Alpha level was 0.05 in all statistical tests.

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**Competing interests**

The authors declare no competing or financial interests.

**Author contributions**

A.D.K. and D.A.P. designed the study, A.D.K. performed the experiments, and A.D.K. and D.A.P. analyzed the data and wrote the paper.

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**Supplementary material**

Supplementary material available online at http://dmm.biologists.org/lookup/suppl/doi:10.1242/dmm.018804/-/DC1

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