Gaucher disease (GD) is an autosomal recessive lysosomal storage disorder caused by mutations in the glucosidase, beta, acid (GBA) gene that encodes the lysosomal enzyme glucosylceramidase (GCase). GCase deficiency leads to characteristic visceral pathology and, in some patients, lethal neurological manifestations. Here, we report the generation of mouse models with the severe neuroopathic form of GD. To circumvent the lethal skin phenotype observed in several of the previous GCase-deficient animals, we genetically engineered a mouse model with strong reduction in GCase activity in all tissues except the skin. These mice exhibit rapid motor dysfunction associated with severe neurodegeneration and apoptotic cell death within the brain, reminiscent of neuroopathic GD. In addition, we have created a second mouse model, in which GCase deficiency is restricted to neural and glial cell progenitors and progeny. These mice develop similar pathology as the first mouse model, but with a delayed onset and slower disease progression, which indicates that GCase deficiency within microglial cells cannot rescue this neurodegenerative disease. These mouse models have significant implications for the development of therapy for patients with neuroopathic GD.


The authors declare no conflict of interest.

Acknowledgments: GCase, glucosylceramidase EC 3.2.1.21 (acid β-glucosidase); GD, Gaucher disease; Glicer, glucosylceramide; GBA, glucosidase, acid, beta; Inl, loxP-neo-loxP.

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Murine models of acute neuronopathic Gaucher disease


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viable, but with pathology restricted to the visceral tissues (17, 19, 20, 23), the K14-lnl/lnl mice developed a rapidly progressing neurological disease after an initial symptom-free period of ~10 days. The K14-lnl/lnl mice showed symptoms of motor dysfunction including abnormal gait, hyperextension of the neck, and seizures (SI Movie 1), which are also common signs of acute neuronopathic GD (8, 9). The mice developed continuous seizures and were killed at 2 weeks of age, at end-stage paralysis (Fig. 1B).

K14-lnl/lnl mice had smaller brains than control animals (data not shown). All brain regions were present and had normal gross anatomy (Fig. 2A–H), indicating that GCase does not have a fundamental role in the formation of the brain. Microscopic analysis, however, revealed a substantial reduction in cellular density throughout the brain, with some regions such as the cortex and thalamus being particularly affected (Fig. 2F–H). The most prominently involved cortical layer (layer V) showed a profound loss of large pyramidal neurons (Fig. 2G), as reported in patients with type 2 GD (11, 12, 15). The cerebellum and nuclei of the pons and medulla also displayed a considerable reduction in the number of cells (SI Fig. 7). The reduction in cellular density was associated with abundant pyknotic nuclei (Fig. 2I). Large neurons were frequently surrounded by ameboid-shaped cells, which had the typical morphology of phagocytic microglia cells (Fig. 2J). Some neurons, especially in the motor trigeminal nuclei and pons region, had huge vacuoles within their cell bodies, suggesting neuronal accumulation of lipids because of GCase deficiency (Fig. 2K and L). Similar neuronal vacuoles have been observed in patients (11). A marked increase in apoptotic cell death was observed in various regions of the brain, for example, in the thalamus, the dendate gyrus of the hippocampus, and the cerebellum in GCase-deficient mice compared with control animals as measured by TUNEL and activated caspase 3 (Fig. 3A–L). We also observed increased labeling for TUNEL and activated caspase 3 in medulla and pons region (data not shown). Moreover, strong
FluoroJade B labeling of pyramidal neurons was selectively detected in cortical layer V of the K14-lnl/lnl brain, which further demonstrated neurodegeneration and indicated a differential vulnerability in neurons to the toxicity of the deficiency of GCase (Fig. 3N). Similarly, neurons of CA3 and dentate gyrus regions underwent degeneration in the hippocampus of animals with...
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K14-lnl/lnl mice and Nestin-flox/flox mice were analyzed at end-stage paralysis, corresponding to an age of 3 weeks, respectively. (Scale bars: A, 600 μm; B–E, 80 μm; F, 20 μm. Scale within columns is uniform.)

Reduced GCase Activity (Fig. 3O), whereas the CA1 region was unaffected, again reflecting findings in type 2 GD patients (15). The calbindin marker for Purkinje neurons revealed a partial loss of these cells in the cerebellum and a profound swelling of their axons (Fig. 3 P and Q). The presence of activated caspase 3-stained cells with a typical neuronal morphology further confirmed the degeneration of neurons in the mutant mice (Fig. 3R). Oil red O staining revealed a substantial accumulation of lipid in both neuronal cell bodies and axons of the K14-lnl/lnl brains (Fig. 3 T, U, W, and X). We also observed a selective accumulation of lipid in the Purkinje cells of the cerebellum (Fig. 3 W and X). In contrast, control mice did not display specific oil red O staining (Fig. 3 S and V). In addition, massive astrogliosis and microglial proliferation, visualized by GFAP and Iba-1 immunostaining, respectively, were present in regions with the most pronounced neuronal loss (SI Fig. 8). These findings demonstrate that the K14-lnl/lnl mice exhibit severe neurodegenerative pathology similar to patients with acute neuromopathic GD (2, 10–16).

Mice with Neuronal and Macroglial GCase Deficiency, but Normal Microglial GCase Activity, Develop Signs Similar to K14-lnl/lnl Mice, but with Slower Progression. It is not known whether the severe neurodegenerative phenotype of type 2 GD is caused by GCase deficiency in neuronal cells, or whether GCase-deficient microglia/macrophages that are of hematopoietic origin (24, 25) play an important role in the pathogenesis of this condition. To restrict GCase deficiency to the progeny of neural and glial cell precursors while maintaining normal enzyme activity within microglial cells, we crossed Nestin-Cre (26) mice into gba{	extsuperscript{flox/flox}} mice, creating gba{	extsuperscript{flox/flox}}, Nestin-Cre mice (referred to as Nestin-flox/flox throughout the text; SI Fig. 9). The Nestin-flox/flox mice were indistinguishable from littermates at birth but eventually developed signs similar to the K14-lnl/lnl mice. The onset of these signs was delayed in Nestin-flox/flox mice compared with K14-lnl/lnl mice (2–3 weeks vs. 10 days, respectively). The neurological abnormalities included rigidity of limbs and abnormal gait, which eventually led to constant seizures and paralysis (defined as end stage) on average 1 week later in the Nestin-flox/flox mice than the K14-lnl/lnl mice (SI Fig. 9). The progression from onset of symptoms to end-stage paralysis was slower in the Nestin-flox/flox mice compared with the K14-lnl/lnl mice (7–10 days vs. 3–4 days, respectively), suggesting that although GCase deficiency within microglia is not the primary contributor to the neurodegeneration in our mouse models, they might influence the onset and progression of the disease. At end-stage paralysis, the Nestin-flox/flox mice demonstrated a severe reduction in GCase activity in the brain compared with spleen, liver, and gba{	extsuperscript{flox/flox}}, Nestin-Cre mice (hereafter referred to as Nestin-wt) (SI Fig. 9). Increased Glycer was observed in brain tissue of Nestin-flox/flox mice but not in tissues from control mice (Nestin-wt and Nestin-lnl/wt) (SI Fig. 9).

GCase Activity Is Critical for Neuronal Survival. Similar to K14-lnl/lnl, Nestin-flox/flox mice developed massive neuronal loss, astrogliosis (data not shown), and microgliosis (Fig. 4). The most striking difference between these mutant mice appeared at the level of microglial activation (Fig. 4). By using microglial markers Iba-1 and mac-2, substantial changes in size, number, and morphology of microglia cells was clearly evident in various brain regions such as cortex, thalamus, and cerebellum in both K14-lnl/lnl and Nestin-flox/flox mice (Fig. 4). Because the nestin promoter is active in neuronal and glial progenitors and microglia are of hematopoietic origin (24, 25), microglial cells in Nestin-flox/flox mice were expected to have normal GCase activity. GCase activity in hematopoietic cells from the Nestin-flox/flox mice was, indeed, normal (data not shown), strongly indicating normal GCase activity in microglial cells. Moreover,
higher levels of GlcCer were present within the brains of K14-lnl/lnl mice compared with Nestin-flox/flox brains and, importantly, lipid-engorged microglia cells could only be found in K14-lnl/lnl animals (Figs. 1F, 4F and R, and SI Fig. 9), further supporting normal GCase activity in microglial cells of the Nestin-flox/flox brain. As expected, we observed activation of microglia in brain regions associated with more-severe cell death in both K14-lnl/lnl and Nestin-flox/flox mice. Interestingly, Nestin-flox/flox mice exhibited a more profound activation and proliferation of microglial cells than K14-lnl/lnl mice (Fig. 4), suggesting that GCase deficiency within microglial cells decreased the level of microglia proliferation and/or activation in response to the considerable cell death. Together, these results show that GCase activity is critical for neuronal survival and indicate that loss or reduction of GCase activity in microglia is likely responsible for the more-rapid progression of neuropathology in K14-lnl/lnl mice compared with Nestin-flox/flox mice.

Discussion

In this study, we describe the development of two mouse models of the severe neuropathologic form of GD. The disease phenotype in the K14-lnl/lnl mouse, in which the GCase deficiency is caused by abnormal splicing of the gba transcript, shows a close resemblance to patients with type 2 GD in terms of clinical signs and histopathological findings within the CNS. Similar to GD patients, we also found elevated quantities of the second substrate for GCase; glucosylsphingosine in the brain of the K14-lnl/lnl mouse (data not shown). Previous studies have revealed that accumulation of glucosylsphingosine results in neuronal toxicity (27, 28). Our mouse models have implications in the investigation of pathogenic mechanisms that are involved in the CNS manifestations of type 2 GD.

A frequent finding in the brains of patients with type 2 GD is activation and proliferation of microglia (10–13, 29). We observed massive activation of microglia in various regions in the brain of the K14-lnl/lnl mouse. We found several swollen microglia that were almost 100 times the size of normal resting microglia (Fig. 4). Although it has been anticipated that GCase-deficient neurons are responsible for the severe CNS symptoms, this has never been verified. We therefore wished to learn if the massive storage and/or subsequent activation of microglia were responsible for the severe neurological abnormalities in the K14-lnl/lnl mice, because activation of microglia has been shown to be involved in other neurodegenerative disorders (30–33). The Nestin-Cre mice have previously been widely applied to create conditional deletion of genes within the CNS and have been shown to induce effective and widespread recombination in precursors of both neurons and glia, such as astrocytes and oligodendrocytes (26, 34–36). The CNS-restricted GCase deficiency in the Nestin-flox/flox mouse caused a disease phenotype that was similar to the K14-lnl/lnl mouse, although the onset of manifestations was delayed and progression of the disease was slower. Indeed, microglia have recently been reported to affect disease progression in mouse models of neurodegenerative disorders such as amyotrophic lateral sclerosis (32). There was a higher degree of activation and proliferation of microglia within the Nestin-flox/flox brain compared with the K14-lnl/lnl brain demonstrating that these hematopoietically derived cells are likely to function normally in the Nestin-flox/flox mice. We cannot, however, exclude the possibility that alterations of GCase activity in other nonneuronal cells may also contribute to the observed neurodegenerative phenotype. The results suggest that although GCase-deficient microglia cells are not primary determinants of the CNS pathogenesis in GCase deficient mice, they contribute by influencing the onset and progression of the disease. Our data suggest that restoration of GCase activity in cells of hematopoietic origin (including microglia) through conventional bone marrow transplantation might slow disease progression but is unlikely to cure GD patients with severe CNS involvement. It is possible that overexpression of GCase within hematopoietic stem cells after gene transfer may be more effective than transplantation of unmodified cells, but this needs to be thoroughly tested. It is not clear whether gene therapy of a proportion of the cells in the CNS will result in a clinical benefit. Selection of an appropriate GCase vector, delivery method, and target cells will require careful consideration to achieve total correction of the disease.

In conclusion, our mouse model (K14-lnl/lnl) shows high similarity, both in pathological findings and clinical manifestations, to patients with severe neuronopathic GD. Our study also indicates that GCase deficiency in microglial cells is not the primary determinant in the CNS pathogenesis of GD but may influence disease onset and progression. It is anticipated that both of the models generated in this study will be valuable in investigations on the pathogenesis as well as in the development of therapy for patients with neuronopathic GD.

Materials and Methods

Generation of Transgenic Mice. A detailed description of the generation of the mice in this study and genotype screening through PCR is available in SI Materials and Methods. The K14-Cre mice (21) were interbred to generate gbafloxflox−/−; K14-Cre mice (referred to as K14-lnl/lnl throughout the text). Control animals gbafloxflox−/−; Nestin-Cre and gbafloxflox−/−; K14-Cre were referred to as K14-lnl/wt and K14-wt, respectively. Gba floxflox mice (20) were crossed with Nestin-Cre mice (26) (a kind gift from Klas Kullander, Uppsala University, Uppsala, Sweden) to generate gbafloxflox−/−; Nestin-Cre mice (referred to as Nestin-flox/wt) and gbafloxflox−/−; Nestin-Cre (referred to as Nestin-wt).

Histochemical Analysis. Information on tissue preparatory procedures is available in SI Materials and Methods. The following primary antibodies were used: rabbit Ab anti-GFAP (1:1,000; Dako, Glostrup, Denmark), rabbit Ab anti-Iba-1 (1:1,000; Wako, Osaka, Japan), rabbit Ab anti-calbindin D-28 (1:500; Swant, Bellinzona, Switzerland), rabbit anti-cleaved caspase 3 (1:100; Cell Signaling Technology, Beverly, MA), and rat Ab anti-mac-2 (1:500; Cedarlane, Ontario, Canada). Apoptotic cells were also detected with TUNEL assay by using ApopTag peroxidase in situ kit (Chemicon, Temecula, CA). FluorJade B (Chemicon) and Nissl stainings were performed as described previously (37). Lipid accumulations in brain slices were revealed with 0.5% oil red O (Sigma, Stockholm, Sweden) in 60% isopropanol.

RT-PCR, cDNA Sequencing, and Q-RT-PCR. A detailed description of RT-PCR and sequencing procedures are available in SI Materials and Methods.

Substrate Accumulation Analysis and Histopathological Studies. Information concerning these procedures is available in SI Materials and Methods. The glucosylceramide content was determined as previously described (38). Fixed, sectioned tissue was stained with hematoxylin-eosin or periodic acid/Schiff (PAS) for microscopic examination.

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Other tissues: "floxed allele"

Neuronal precursors: "null allele"

Nestin-flox/flox

Nestin-wt

Relative GCase activity

0 0.5 1.0 1.5

brain spleen liver

K14-lnl/lnl Nestin-flox/flox

Percent survival

0 10 20 30 40

Days

Nestin-flox/flox control

Glucos (nmoles/mg protein)

0 2 4 6

brain spleen liver