

Animal models for Gaucher disease research

Tamar Farfel-Becker^{1,*}, Einat B. Vitner^{1,*} and Anthony H. Futerman^{1,‡}

Gaucher disease (GD), the most common lysosomal storage disorder (LSD), is caused by the defective activity of the lysosomal hydrolase glucocerebrosidase, which is encoded by the *GBA* gene. Generation of animal models that faithfully recapitulate the three clinical subtypes of GD has proved to be more of a challenge than first anticipated. The first mouse to be produced died within hours after birth owing to skin permeability problems, and mice with point mutations in *Gba* did not display symptoms correlating with human disease and also died soon after birth. Recently, conditional knockout mice that mimic some features of the human disease have become available. Here, we review the contribution of all currently available animal models to examining pathological pathways underlying GD and to testing the efficacy of new treatment modalities, and propose a number of criteria for the generation of more appropriate animal models of GD.

Introduction

Gaucher disease (GD), the most common lysosomal storage disorder (LSD), is caused by the defective activity of glucosylceramidase (GlcCerase; also known as glucocerebrosidase), the lysosomal hydrolase that is responsible for the degradation of glucosylceramide (GlcCer) (Vitner et al., 2010a). As a result of the autosomal recessive genetic defect, GlcCer and glucosylsphingosine (GlcSph) accumulate intracellularly. Tissue macrophages engorged with glycolipid-laden lysosomes (known as 'Gaucher cells') are the hallmark of the disease.

GD has traditionally been divided into three subtypes on the basis of the age of onset and of signs of nervous system involvement. Type 1, the non-neuronopathic form, is the most common (>90% of patients), and major symptoms include enlargement of the spleen and liver (hepatosplenomegaly), thrombocytopenia, anaemia and skeletal disease (Mistry and Zimran, 2007). In the neuronopathic forms [types 2 and 3; both also referred to as neuronal GD (nGD)], which are much more rare than type 1, neurological abnormalities are observed in addition to visceral symptoms. Patients with the

acute neuronopathic form (type 2) display neurological signs before 6 months of age and normally die at 2-4 years of age (Schiffmann and Vellodi, 2007); a perinatal lethal form is also found, in which babies present with erythematous and shiny abnormal skin (the 'collodion baby' phenotype) (Schiffmann and Vellodi, 2007; Gupta et al., 2010). The subacute, chronic neuronopathic form (type 3) displays rather similar symptoms to type 2 GD but with a later onset and decreased severity. Almost 300 mutations have been reported in the *GBA* gene (the gene encoding GlcCerase) (Hruska et al., 2008). Genotype-phenotype correlations are generally poor: some patients with the same mutation show widely different phenotypes, although certain mutations do predispose to a particular disease type.

Recent studies have shown an association between individuals with type 1 GD and/or GD carriers and Parkinson's disease (Box 1). Together with observations of several subclinical neurological manifestations in type 1 GD patients (Cherin et al., 2010), it has been suggested that the traditional classification of GD into three clinical phenotypes might need to be re-evaluated. It has been proposed that GD should be considered a continuum of clinical manifestations rather than distinct subtypes (Sidransky, 2004).

Currently available treatments for GD include enzyme replacement therapy (ERT) and substrate reduction therapy (SRT). Although ERT has a marked effect on non-neurological manifestations, it has no demonstrable effect on neurological abnormalities because the enzyme does not cross the blood-brain

Box 1. Linking Parkinson's disease with GD and other LSDs using GD mouse models

There has been significant recent interest in GD among neurologists owing to observations suggesting a genetic link between GD and Parkinson's disease (PD) (Sidransky et al., 2009). Several studies have used some of the mouse models described in this Commentary to try to tease out the mechanistic connection between these two diseases. One approach is to look for changes in the level or distribution of α -synuclein, a protein that is known to form aggregates in PD. Notably, a single injection of CBE to a mouse results in increased nigral α -synuclein levels after 48 hours (Manning-Bog et al., 2009). In addition, α -synuclein aggregation was observed in brains from PS-NA mice and in D409H/D409H mice, but not in Kn-9H mice (Xu et al., 2011a) (see Box 2), and there was a difference in α -synuclein concentrations in mice carrying one versus two D409V alleles (Cullen et al., 2011). Progressive accumulation of proteinase-K-resistant α -synuclein-ubiquitin aggregates in hippocampal neurons together with a coincident memory deficit was also detected in D409V/D409V mice (Sardi et al., 2011), and α -synuclein accumulation was observed in a 4L/PS-NA mouse (see Box 2) (Mazzulli et al., 2011). However, α -synuclein aggregates have also been observed in the brains of mouse models of other LSDs, and case studies and other clinical data suggest a more general link between PD and LSDs (Shachar et al., 2011). Collectively, this suggests that the search for biochemical and cellular pathways that link PD with GD should not be limited exclusively to changes that occur in GD, but rather should include changes that might be common to a variety of animal models of LSDs.

¹Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel

*These authors contributed equally to this work

‡Author for correspondence (tony.futerman@weizmann.ac.il)

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barrier (Vellodi et al., 2009). Other potential treatments that hold promise for the future include cell and gene therapy as well as pharmacological chaperones (Enquist et al., 2006).

Until recently, little was known about the molecular mechanisms linking GlcCer accumulation to cellular dysfunction and disease, in part owing to problems associated with generating animal models that faithfully mimic GD symptoms. In this Commentary, we describe attempts to generate GD animal models. For each model, we briefly discuss how it was generated, provide information about research performed using the model, summarize its contribution to the field, and highlight its particular advantages and disadvantages (summarized in Fig. 1).

Chemically induced models of GD

The first attempt to generate an animal model for use in GD research involved administration of a GlcCerase inhibitor, conduritol- β -epoxide (CBE) (Kanfer et al., 1975), to mice. Daily intraperitoneal injections of CBE for 3 weeks resulted in >90% inhibition of GlcCerase activity and accumulation of GlcCer in the spleen, liver and brain that could be reversed upon cessation of CBE treatment (Stephens et al., 1978). Further augmentation of GlcCer levels was achieved by injecting mice with a mixture of CBE and GlcCer (Datta and Radin, 1986; Datta and Radin, 1988; Marshall et al., 2002), a model that was also used to demonstrate the success of gene therapy for normalizing GlcCer levels in Kupffer cells after transduction of hepatocytes with the gene encoding human GlcCerase (Marshall et al., 2002). Young mice that received eight or nine daily injections of CBE developed severe neurological symptoms and died 7–12 days after the final CBE injection, suggesting that lesions in the brain might not be reversible and emphasizing the need for early therapeutic intervention in nGD (Xu et al., 2008). Although this chemically induced model has not been widely used, it is a relatively quick and inexpensive model that could potentially be used more extensively to examine neurological defects and to test the efficacy of novel therapies.

The *Gba*-knockout mouse

The first genetic model of GD was produced in 1992 (Tybulewicz et al., 1992) by insertion of a *Neo* cassette in exons 9 and 10 of the *Gba* gene, which created a mouse carrying a null mutation resulting in <4% GlcCerase activity compared with wild-type mice. This resulted in a very robust GD phenotype, mimicking severe type 2 patients (Sidransky et al., 1992). GlcCer accumulation was observed in liver, brain and lungs, and in the lysosomes of spleen and liver macrophages. The neonatal lethality of these *Gba*^{-/-} mice occurred owing to markedly elevated trans-epidermal water loss (Sidransky et al., 1992; Holleran et al., 1994) caused by permeability barrier defects in the skin (Holleran et al., 1994); this established a previously unappreciated role for GlcCer in skin formation, which was later recognized as a distinct human GD phenotype (Sidransky et al., 1992).

Despite the initial excitement when the *Gba*^{-/-} mouse was generated, it has proved less useful than originally anticipated owing to its short lifespan. However, some studies were performed using *Gba*^{-/-} mice to examine limited aspects of neuropathology (Willemsen et al., 1995). For instance, the *Bcl2* gene was found to be downregulated in *Gba*^{-/-} brain (Hong et al., 2004), as was the expression of brain-derived neurotrophic factor (BDNF) and nerve

growth factor (NGF) (Kim et al., 2006). By contrast, the expression of some pro-inflammatory cytokines and reactive oxygen species was elevated (Hong et al., 2006).

Mouse models of GD based on point mutations

After generation and characterization of the *Gba*^{-/-} mouse, a number of attempts were made to create less severe mouse models of GD. The mouse and human genes share 82% identity (O'Neill et al., 1989), so an obvious strategy was to generate mice with the same mutations known to cause the human disease, such as L444P, which predisposes individuals to neuronopathic forms of GD, and N370S, which is the most common mutation causing type 1 GD. A number of mouse strains have been generated using this approach, although their usefulness has been somewhat mixed.

RecNcil and L444P/L444P mice

Mice carrying the recombinant allele traditionally known as the 'RecNcil' mutation (L444P/A456P), which causes type 2 disease in humans, and mice homozygous for the L444P mutation (L444P/L444P mice) were generated using the single insertion mutagenesis procedure (Liu et al., 1998). Mice carrying the RecNcil mutation developed more severe disease than those that were homozygous for L444P: RecNcil mice exhibited GlcCerase activity in the liver, brain and skin that was <4–9% that of wild-type mice, whereas GlcCerase activity was ~20% in L444P/L444P mice compared with wild type. Both strains were abnormal at birth, with red and wrinkled skin; RecNcil mice displayed a more severe phenotype (smaller size and no milk in their stomachs). Surprisingly, both strains died soon after birth (Liu et al., 1998) owing to compromised skin barrier function rather than to neurological defects. The RecNcil mouse was subsequently used to study the role of GlcCerase in epidermal function (Doering et al., 1999).

The lifespan of the L444P/L444P mouse was improved by crossing L444P^{+/-} mice with a mouse bearing a knockout in the gene encoding GlcCer synthase (*Ugcg*) (Mizukami et al., 2002), the protein that is responsible for the synthesis of GlcCer, generating a *Gba*^{+L444P};*Ugcg*^{+/-} mouse. A series of intercrosses of *Gba*^{+L444P};*Ugcg*^{+/-} mice gave rise to adult mice that were L444P/L444P for the gene encoding GlcCerase and wild type for the *Ugcg* gene; these are referred to as *Gba*^{L444P/L444P};*Ugcg*^{+/+} mice. Although these mice seem to have the same genotype as the previously described L444P/L444P mice, there is a distinct difference in their phenotype and disease severity, which could be due to the genetic background or to strain differences. Approximately 50% of the mice survive for up to a year, and GlcCerase activity is 15–20% of wild-type activity, similar to levels found in type 1 human GD patients. The mice display some features of GD, such as systemic inflammation, anaemia, skin abnormalities and depressed serum cholesterol levels (Mizukami et al., 2002). However, GlcCer does not accumulate, Gaucher cells are not detected and other features that are typical of GD are not observed (Mizukami et al., 2002). Nevertheless, these mice have been used to study the efficacy of a chemical chaperone as a treatment for GD (Khanna et al., 2010), which resulted in a significant increase in GlcCerase activity in a number of tissues. These mice have also been used as a source of cultured neurons (Farfel-Becker et al., 2009; Kacher and Futerman, 2009).

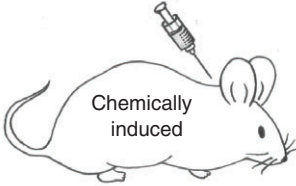
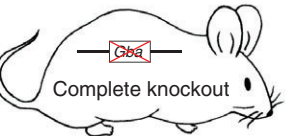
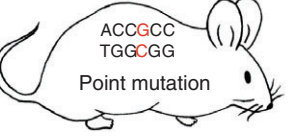
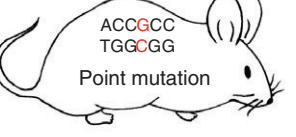
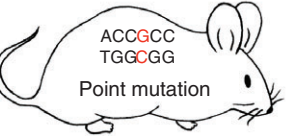
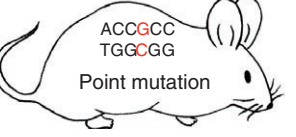
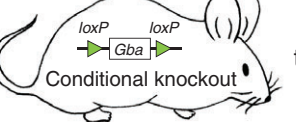
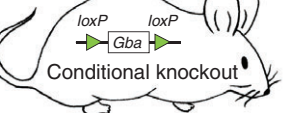
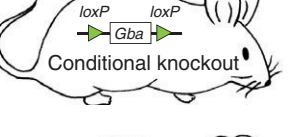
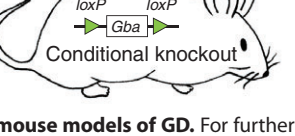
	Mouse	Main features	Use in GD research	Pros	Cons
1975	CBE-induced (Kanfer et al., 1975)	 Chemically induced	Efficacy of gene therapy	Can manipulate dose and time; mimics neurological signs	Non-genetic model
1992	Gba^{-/-} (Tybulewicz et al., 1992)	 Complete knockout	Involvement of GlcCerase in skin formation	Paved the way for creation of more useful mouse models	Die within 24 hours of birth
1998	RecNcil (L444P/A456P and L444P) (Liu et al., 1998)	 Point mutation ACCGCC TGGCGG	Involvement of GlcCerase in skin formation	Cell culture experiments	Die soon after birth
2002	Gba^{L444P/L444P}; Ugcg^{+/+} (Mizukami et al., 2002)	 Point mutation ACCGCC TGGCGG	Therapeutic evaluation targeting the mutant enzyme	Long lifespan; cell culture experiments	No GlcCer accumulation; no Gaucher cells; does not mimic human phenotype
2003	V394L, D409H and D409V (Xu et al., 2003)	 Point mutation ACCGCC TGGCGG	Testing therapeutic options	Long lifespan; cell culture experiments	No phenotypic abnormalities; no GlcCer accumulation in the brain
2003	N370S (Xu et al., 2003)	 Point mutation ACCGCC TGGCGG	-	Cell culture experiments	Die soon after birth
2006, 2010	Mx1-Cre-loxP (Enquist et al., 2006; Mistry et al., 2010)	 Conditional knockout	Cell and gene therapy; mechanistic basis for osteopenia	Normal life span; model for type 1 GD	The genetic mutation does not mimic a real mutation as seen in patients
2007	Nestin-flox/flox (Enquist et al., 2007)	 Conditional knockout	Role of microglia in nGD; progression of neuropathological changes	Reminiscent of neuropathological findings in nGD patients	Die within 3 weeks; GlcCer accumulation only in brain
2007	K14-In1/In1 (Enquist et al., 2007)	 Conditional knockout	Efficacy of ICV-delivered GlcCerase	Reminiscent of neuropathological findings in nGD patients	Die within 2 weeks
2008	Kn-9H mice (Xu et al., 2008)	 Conditional knockout	-	Reminiscent of neuropathological findings in nGD patients	Die within 2 weeks

Fig. 1. A timeline of the generation of mouse models of GD. For further details, see text.

N370S, V394L, D409H and D409V point mutations

No gross phenotypic abnormalities were observed in mice bearing the D409H, D409V or V394L mutations in *Gba*, even though residual GlcCerase activity was only 4–10% of that in wild-type mice. Low levels of GlcCer accumulation and some storage cells were detected in the spleen of these mice, but hepatosplenomegaly was not observed (Xu et al., 2003). Compared with other tissues in these mice, GlcCerase activity was higher in the brain (25%), with no GlcCer accumulation. These mice have been used to test the efficacy of various experimental treatments. D409H/D409H and V394L/V394L mice were used to evaluate the ability of L-type calcium channel blockers such as diltiazem to enhance folding of GlcCerase in the endoplasmic reticulum (Mu et al., 2008; Rigat and Mahuran, 2009), as were fibroblasts derived from these mice. Whereas diltiazem enhanced GlcCerase activity in fibroblasts, it had no effect in the mice (Sun et al., 2009), a finding that emphasizes the importance of complementing studies in cultured cells with studies involving live mice. The GD phenotype of V394L, D409H and D409V mice has also been enhanced by injection with CBE (Xu et al., 2008), and these strains have also been crossed with prosaposin-deficient mice (Box 2).

In contrast to V394L, D409H and D409V mice, N370S mice die within 24 hours of birth (Xu et al., 2003) owing to a skin defect, similar to *Gba*^{-/-} mice. In humans, the N370S mutation is associated with type 1 disease, but up to two-thirds of individuals carrying this mutation seem to be totally asymptomatic (Grabowski, 1997). The skin phenotype in the N370S mice was not found to correlate with the levels of residual GlcCerase activity, implying that there is a key difference in the way that mice and humans degrade GlcCer in the skin (Xu et al., 2003).

A greater reduction in GlcCerase activity was obtained by crossing mice with point mutations with mice carrying null mutations, resulting in a more rapid and earlier development of storage cells and more extensive GlcCer accumulation; however, pathology and storage were not observed in the brain (Xu et al., 2003). For instance, D409V/null mice exhibited ~5% of normal GlcCerase activity, developed Gaucher cells in the liver, spleen, lung and bone marrow by 4 months of age (Xu et al., 2003), and accumulated 4- to 16-fold greater levels of GlcCer than wild-type mice (McEachern et al., 2006). These mice have been used to test several therapeutic options, such as adeno-associated viral (AAV)-mediated gene therapy to deliver the human GlcCerase cDNA, which prevented disease development and corrected GlcCer accumulation in visceral organs (McEachern et al., 2006), administration of a potential molecule for use in SRT (McEachern et al., 2007) both by itself and in combination with ERT (Marshall et al., 2010), and the potential use of two new recombinant GlcCerase proteins (Xu et al., 2010). GD pathophysiology has also been explored (Xu et al., 2011b) in these mice by using genome-wide mRNA microarrays and immunohistochemistry of lung and liver, which suggested roles for the macrophage-activation response, including interferon- γ -regulated pro-inflammatory and interleukin-4-regulated anti-inflammatory networks (Xu et al., 2011b) in pathology.

Conditional GD mice

Type 1 GD models

A major breakthrough in the development of GD mouse models was the generation, in 2006, of a conditional GD mouse. In general,

Box 2. Use of prosaposin-deficient mice in GD research

Saposins are sphingolipid-activator proteins that are required for the lysosomal hydrolysis of sphingolipids and glycosphingolipids. Saposins A–D are derived from the same precursor, prosaposin. Each saposin is involved in the degradation of a different sphingolipid. Saposin A facilitates galactosylceramide degradation, saposin B facilitates degradation of sulfatide and other glycolipids, saposin C facilitates GlcCer degradation and saposin D facilitates ceramide degradation (Hoops et al., 2007). A defect in a specific saposin results in accumulation of these sphingolipids, whereas a deficiency in prosaposin leads to accumulation of multiple sphingolipids and neurological disease, and is neonatally fatal (Fujita et al., 1996). Mice that were partially deficient for saposins (termed PS-NA mice) were created by expressing the prosaposin transgene at subnormal levels in the total prosaposin-deficient mouse, which delayed neurological disease compared with the prosaposin-deficient mouse (Sun et al., 2002). PS-NA mice were subsequently crossed with mice carrying the GD-associated point mutations V394L/V394L or D409H/D409H, with the resultant mice designated 4L/PS-NA and 9H/PS-NA, respectively. These mice displayed elevated GlcCer levels in visceral organs and in the brain, and the brain pathology was similar to that seen in the PS-NA mouse, but no hepatosplenomegaly occurred (Sun et al., 2005). Although these mice do not seem to be a classical GD model, they have nevertheless been used in a number of studies, such as the demonstration of the ability of recombinant GlcCerase to reduce GlcCer levels in visceral organs but not in brain (Sun et al., 2006), and to test the relationship between GD and Parkinson's disease (Xu et al., 2011a) (Box 1). Mice with a specific deficiency in saposin C have also been generated (Sun et al., 2010a) and display neurological disease; however, GlcCerase levels were only reduced by ~50% and no GlcCer accumulation was observed, suggesting that the neurological defects in these mice might be due to another role of saposin in the CNS (Sun et al., 2010a; Yoneshige et al., 2010). Mice carrying point mutations in *Gba* (V394L/V394L) have also been crossed with saposin-C-deficient mice, but Gaucher cells were not observed and GlcCer levels were only moderately increased (Sun et al., 2010b). Although the neurological disease observed in these mice supports the idea that the deficiency in saposin C itself, rather than changes in GlcCer levels, is responsible for the phenotype (Sun et al., 2010a), it was used to implicate aberrant axonal autophagy in nGD (Sun and Grabowski, 2010) and to test the in vivo effects of the chaperone isofagomine on pathology (Sun et al., 2011). All in all, the relevance of these various saposin- and prosaposin-deficient mice to the study of GD pathology is a matter of debate.

these mice recapitulate GD clinical symptoms in a much more rigorous manner than the models that preceded them. The first conditional mouse to be generated was a type 1 GD mouse, created using the *Mx1-Cre-loxP* system, allowing Cre-mediated deletion of a floxed *Gba* gene upon treatment with polyinosinic-polycytidylic acid (which activates the *Mx1* promoter) (Enquist et al., 2006). Administration of polyinosinic-polycytidylic acid after birth allowed maintenance of GlcCerase activity during development, thereby avoiding disruption of skin barrier formation. The *Mx1-Cre*-mediated deletion occurred with high efficiency in haematopoietic stem cells and in all progeny cells, including macrophages, but only to a limited extent in the central nervous system (CNS), resulting in a ~100-fold increase in GlcCer levels, the appearance of Gaucher cells in the bone marrow, spleen and liver but not in the brain, and splenomegaly (~tenfold). The mice had a normal lifespan and did not show any gross signs of bone disease or CNS abnormalities (Enquist et al., 2006). These mice have proved useful in showing that both bone marrow transplantation and gene therapy can eliminate Gaucher cells (Enquist et al., 2006), and that only a modest

increase in GlcCerase activity is required to correct pathology (Enquist et al., 2009).

Two other similar conditional models of type 1 GD have been generated. In the first, GlcCerase was specifically deleted in haematopoietic and endothelial cells by use of a mouse line expressing Cre under the *Tie2* promoter (Sinclair et al., 2007), resulting in 7–18% residual GlcCerase activity in liver, spleen, brain, bone marrow and leukocytes, as well as GlcCer accumulation and splenomegaly (Sinclair et al., 2007). A second mouse model was recently generated (Mistry et al., 2010) by using the *Mx1-Cre-loxP* system to conditionally knock out GlcCerase; such mice showed a >95% reduction of GlcCerase activity in cells of the haematopoietic and mesenchymal stem cell lineages, and a dramatic increase in GlcCer and GlcSph levels in spleen and liver. In addition to visceral and haematological pathologies (such as splenomegaly and anaemia), these mice exhibited profound skeletal defects, including focal osteonecrosis and osteopenia, both of which have been reported in type 1 GD patients (Mistry et al., 2010). A number of novel observations were made using this mouse model: osteopenia was determined to arise from an osteoblastic defect in bone formation rather than from increased bone resorption (Mistry et al., 2010); GlcSph and GlcCer inhibited protein kinase C (PKC)-mediated osteoblast proliferation and early differentiation, probably contributing to the bone formation defect; and widespread aberrations in thymic T-cell and dendritic cell development (Mistry et al., 2010) were detected.

Neuronal GD models

A conditional mouse model of acute nGD (type 2 GD) was generated by insertion of a *loxP-Neo-loxP* (Inl) cassette into *Gba* intron 8, and *gba*^{Inl/Inl} mice were then bred with keratin-14-Cre transgenic mice (to create K14-Inl/Inl mice) in which Cre recombinase expression is driven by the K14 promoter, allowing excision of the Inl cassette and restoration of normal GlcCerase levels in the skin (Enquist et al., 2007). K14-Inl/Inl mice had significantly reduced GlcCerase levels in the brain, spleen and liver, a concomitant increase in GlcCer levels, and the appearance of Gaucher cells. After an initial symptom-free period of ~10 days, K14-Inl/Inl mice developed rapidly-progressing neurological disease, leading to continuous seizures and paralysis at 2 weeks of age, which was associated with neuronal loss and microglia activation and proliferation. Some of these features are reminiscent of neuropathological findings in nGD patients (Wong et al., 2004; Enquist et al., 2007). The importance of this mouse cannot be emphasized enough, because it was the first genetically induced mouse model that faithfully recapitulated neuropathological and biochemical aspects of nGD, paving the way for investigation of basic pathogenic mechanisms and potential treatments. For instance, three intracerebroventricular (ICV) injections of recombinant human GlcCerase reduced GlcCer and GlcSph levels, improved neurodegeneration and astrogliosis, and caused an increase in lifespan (from 14 to 23 days) (Cabrera-Salazar et al., 2010). A single ICV injection of an AAV1 vector encoding human GlcCerase further prolonged lifespan to 28.5 days (Cabrera-Salazar et al., 2010), indicating that ICV administration of GlcCerase might be a potential therapeutic approach for nGD patients.

Another conditional nGD model was generated by restricting GlcCerase deficiency to the CNS (Enquist et al., 2007). This nestin-

flox/flox model was designed by flanking the *Gba* gene with two *loxP* sites, enabling cleavage of a segment of the gene by Cre recombinase, resulting in a null allele. When driven by the nestin promoter, Cre recombinase expression is limited to neural and glial cells, but is not present in microglia, leading to a specific deficiency of GlcCerase in these cell types. In nestin-flox/flox mice, GlcCer levels were found to be increased in the brain but not in the spleen or liver (Enquist et al., 2007). They developed similar disease symptoms and neuropathology to the K14-Inl/Inl mice but with a later onset of development of neurological symptoms (~16 days versus ~7 days). On the basis of these observations, it was suggested that GlcCerase deficiency in microglia is not the primary cause of nGD (Enquist et al., 2007).

The main advantage of the nestin-flox/flox mouse compared with the K14-Inl/Inl mouse is that it lives somewhat longer, allowing more in-depth analysis of pathogenic mechanisms. Thus, the nestin-flox/flox mouse has been used to exclude activation of the unfolded protein response as a pathogenic mechanism in nGD (Farfel-Becker et al., 2009), to show alterations in the expression and distribution of cathepsins (Vitner et al., 2010b), to examine the onset and progression of neuropathological changes, and to establish that astrogliosis and microglial activation are temporally and spatially correlated with specific and localized neuronal loss (Farfel-Becker et al., 2011). Although this mouse model has proved tremendously useful, its use is still limited, mainly owing to the rapid onset and extreme severity of disease development.

Another potential mouse model of nGD was generated by insertion of a *Neo* cassette into a *Gba* allele containing the D409H point mutation; upon crossing this mouse with the K14-Cre mouse, excision of the *Neo* cassette occurred, producing low activity of GlcCerase in the skin (Xu et al., 2008). These mice, termed Kn-9H, had a similar phenotype to the K14-Inl/Inl mice; intriguingly, their CNS abnormalities were completely mimicked by 10 days of CBE treatment (Xu et al., 2008).

Non-mouse animal models

Two non-mouse GD models have been described. The first, which is no longer available, was an 8-month-old Australian Sydney Silky dog (Hartley and Blakemore, 1973), which had reduced GlcCerase activity (Van De Water et al., 1979), GlcCer accumulation, Gaucher cells and progressive neurological disease (Hartley and Blakemore, 1973). More recently, a naturally occurring nGD sheep was discovered on a Southdown sheep stud farm in Victoria, Australia (Karageorgos et al., 2010). Affected neonatal lambs showed severe neurological disease, principally shaking and an inability to stand, had abnormal and thickened skin at birth, and only lived for a few days. GlcCerase activity was reduced and GlcCer accumulated (100-fold in the brain and 15-fold in the liver). Lysosomal storage was observed in spleen macrophages, lymph nodes and thymus, and pathological changes were observed in the central and peripheral nervous systems (Karageorgos et al., 2010). Mutational analysis identified two homozygous missense mutations, one of which has also been reported in humans with GD. This sheep is the first available nGD animal model resulting from spontaneous mutations rather than from a null allele, and is also the first available large animal model of nGD, which should prove very useful in assessing the efficacy and delivery of putative treatments into and throughout the brain.

Summary and future perspectives

The long journey to generate suitable animal models for use in GD research began almost 4 decades ago and has met several unanticipated challenges along the way, primarily the perinatal lethality of the *Gba* knockout mouse, and differences between human and mouse phenotypes of specific GD mutations. Nevertheless, the mice that have been generated have enabled some progress in understanding the mechanisms of disease pathology and in testing putative novel therapeutic treatments. The recent availability of conditional knockout mouse models that recapitulate, to a large extent, type 1 and nGD symptoms has significantly advanced the field, as will the availability of a large animal (sheep) model. One simple model, which has been somewhat ignored over the past couple of decades, is the chemically induced (CBE) model. On the basis of comparisons with the Kn-9H model, it is now apparent that the neurological symptoms induced by CBE treatment are quite similar to those observed in the Kn-9H mouse model, validating the use of CBE as a means to model nGD in mice. Moreover, by varying the dose and time of CBE injection, it should be possible to generate mice with varying degrees of pathology, and also to examine disease recovery (after stopping CBE treatment).

Although progress in generating GD animal models has accelerated significantly over the past decade or so, the 'perfect' GD mouse model is still lacking. Such a mouse should faithfully recapitulate human disease symptoms; this is particularly true for point mutations. Animal models should also show differing disease severity, have long enough lifespans such that the therapeutic window is sufficient to test new therapies, and, finally, should carry mutations in all tissues and cell types, similar to human patients. We anticipate that such models will become available in the years ahead.

REFERENCES

- Cabrera-Salazar, M. A., Bercury, S. D., Ziegler, R. J., Marshall, J., Hodges, B. L., Chuang, W. L., Pacheco, J., Li, L., Cheng, S. H. and Scheule, R. K.** (2010). Intracerebroventricular delivery of glucocerebrosidase reduces substrates and increases lifespan in a mouse model of neuronopathic Gaucher disease. *Exp. Neurol.* **225**, 436-444.
- Cherin, P., Rose, C., de Roux-Serratrice, C., Tardy, D., Dobbelaere, D., Grosbois, B., Hachulla, E., Jaussaud, R., Javier, R. M., Noel, E. et al.** (2010). The neurological manifestations of Gaucher disease type 1, the French Observatoire on Gaucher disease (FROG). *J. Inher. Metab. Dis.* **33**, 331-338.
- Cullen, V., Sardi, S. P., Ng, J., Xu, Y. H., Sun, Y., Tomlinson, J. J., Kolodziej, P., Kahn, I., Saftig, P., Woulfe, J. et al.** (2011). Acid beta-glucosidase mutants linked to gaucher disease, parkinson disease, and lewy body dementia alter alpha-synuclein processing. *Ann. Neurol.* **69**, 940-953.
- Datta, S. C. and Radin, N. S.** (1986). Glucosylceramide and the level of the glucosidase-stimulating proteins. *Lipids* **21**, 702-709.
- Datta, S. C. and Radin, N. S.** (1988). Normalization of liver glucosylceramide levels in the "Gaucher" mouse by phosphatidylserine injection. *Biochem. Biophys. Res. Commun.* **152**, 155-160.
- Doering, T., Proia, R. L. and Sandhoff, K.** (1999). Accumulation of protein-bound epidermal glucosylceramides in beta-glucocerebrosidase deficient type 2 Gaucher mice. *FEBS Lett.* **447**, 167-170.
- Enquist, I. B., Nilsson, E., Ooka, A., Mansson, J. E., Olsson, K., Ehinger, M., Brady, R. O., Richter, J. and Karlsson, S.** (2006). Effective cell and gene therapy in a murine model of Gaucher disease. *Proc. Natl. Acad. Sci. USA* **103**, 13819-13824.
- Enquist, I. B., Bianco, C. L., Ooka, A., Nilsson, E., Mansson, J. E., Ehinger, M., Richter, J., Brady, R. O., Kirik, D. and Karlsson, S.** (2007). Murine models of acute neuronopathic Gaucher disease. *Proc. Natl. Acad. Sci. USA* **104**, 17483-17488.
- Enquist, I. B., Nilsson, E., Mansson, J. E., Ehinger, M., Richter, J. and Karlsson, S.** (2009). Successful low-risk hematopoietic cell therapy in a mouse model of type 1 Gaucher disease. *Stem Cells* **27**, 744-752.
- Farfel-Becker, T., Vitner, E., Dekel, H., Leshem, N., Enquist, I. B., Karlsson, S. and Futerman, A. H.** (2009). No evidence for activation of the unfolded protein response in neuronopathic models of Gaucher disease. *Hum. Mol. Genet.* **18**, 1482-1488.
- Farfel-Becker, T., Vitner, E. B., Pressey, S. N., Eilam, R., Cooper, J. D. and Futerman, A. H.** (2011). Spatial and temporal correlation between neuron loss and neuroinflammation in a mouse model of neuronopathic Gaucher disease. *Hum. Mol. Genet.* **20**, 1375-1386.
- Fujita, N., Suzuki, K., Vanier, M. T., Popko, B., Maeda, N., Klein, A., Henseler, M., Sandhoff, K. and Nakayasu, H.** (1996). Targeted disruption of the mouse sphingolipid activator protein gene: a complex phenotype, including severe leukodystrophy and wide-spread storage of multiple sphingolipids. *Hum. Mol. Genet.* **5**, 711-725.
- Grabowski, G. A.** (1997). Gaucher disease: gene frequencies and genotype/phenotype correlations. *Genet. Test.* **1**, 5-12.
- Gupta, N., Oppenheim, I. M., Kauvar, E. F., Tayebi, N. and Sidransky, E.** (2010). Type 2 Gaucher disease: phenotypic variation and genotypic heterogeneity. *Blood Cells Mol. Dis.* **46**, 75-84.
- Hartley, W. J. and Blakemore, W. F.** (1973). Neurovisceral glucocerebrosidase storage (Gaucher's disease) in a dog. *Vet. Pathol.* **10**, 191-201.
- Holleran, W. M., Ginns, E. I., Menon, G. K., Grundmann, J. U., Fartasch, M., McKinney, C. E., Elias, P. M. and Sidransky, E.** (1994). Consequences of beta-glucocerebrosidase deficiency in epidermis. Ultrastructure and permeability barrier alterations in Gaucher disease. *J. Clin. Invest.* **93**, 1756-1764.
- Hong, Y. B., Kim, E. Y. and Jung, S. C.** (2004). Down-regulation of Bcl-2 in the fetal brain of the Gaucher disease mouse model: a possible role in the neuronal loss. *J. Hum. Genet.* **49**, 349-354.
- Hong, Y. B., Kim, E. Y. and Jung, S. C.** (2006). Upregulation of proinflammatory cytokines in the fetal brain of the Gaucher mouse. *J. Korean Med. Sci.* **21**, 733-738.
- Hoops, S. L., Koltter, T. and Sandhoff, K.** (2007). Saposin C and other sphingolipid activator proteins. In *Gaucher Disease* (ed. A. H. Futerman and A. Zimran), pp. 67-84. Taylor and Francis Group: Boca Raton, FL.
- Hruska, K. S., LaMarca, M. E., Scott, C. R. and Sidransky, E.** (2008). Gaucher disease: mutation and polymorphism spectrum in the glucocerebrosidase gene (GBA). *Hum. Mutat.* **29**, 567-583.
- Kacher, Y. and Futerman, A. H.** (2009). Impaired IL-10 transcription and release in animal models of Gaucher disease macrophages. *Blood Cells Mol. Dis.* **43**, 134-137.
- Kanfer, J. N., Legler, G., Sullivan, J., Raghavan, S. S. and Mumford, R. A.** (1975). The Gaucher mouse. *Biochem. Biophys. Res. Commun.* **67**, 85-90.
- Karageorgos, L., Lancaster, M. J., Nimmo, J. S. and Hopwood, J. J.** (2010). Gaucher disease in sheep. *J. Inher. Metab. Dis.* **34**, 209-215.
- Khanna, R., Benjamin, E. R., Pellegrino, L., Schilling, A., Rigat, B. A., Soska, R., Nafar, H., Ranes, B. E., Feng, J., Lun, Y. et al.** (2010). The pharmacological chaperone isofagomine increases the activity of the Gaucher disease L444P mutant form of beta-glucosidase. *FEBS J.* **277**, 1618-1638.
- Kim, E. Y., Hong, Y. B., Go, S. H., Lee, B. and Jung, S. C.** (2006). Downregulation of neurotrophic factors in the brain of a mouse model of Gaucher disease; implications for neuronal loss in Gaucher disease. *Exp. Mol. Med.* **38**, 348-356.
- Liu, Y., Suzuki, K., Reed, J. D., Grinberg, A., Westphal, H., Hoffmann, A., Doring, T., Sandhoff, K. and Proia, R. L.** (1998). Mice with type 2 and 3 Gaucher disease point mutations generated by a single insertion mutagenesis procedure. *Proc. Natl. Acad. Sci. USA* **95**, 2503-2508.
- Manning-Bog, A. B., Schule, B. and Langston, J. W.** (2009). Alpha-synuclein-glucocerebrosidase interactions in pharmacological Gaucher models: a biological link between Gaucher disease and parkinsonism. *Neurotoxicology* **30**, 1127-1132.
- Marshall, J., McEachern, K. A., Kyros, J. A., Nietupski, J. B., Budzinski, T., Ziegler, R. J., Yew, N. S., Sullivan, J., Scaria, A., van Rooijen, N. et al.** (2002). Demonstration of feasibility of in vivo gene therapy for Gaucher disease using a chemically induced mouse model. *Mol. Ther.* **6**, 179-189.
- Marshall, J., McEachern, K. A., Chuang, W. L., Hutto, E., Siegel, C. S., Shayman, J. A., Grabowski, G. A., Scheule, R. K., Copeland, D. P. and Cheng, S. H.** (2010). Improved management of lysosomal glucosylceramide levels in a mouse model of type 1 Gaucher disease using enzyme and substrate reduction therapy. *J. Inher. Metab. Dis.* **33**, 281-289.
- Mazzulli, J. R., Xu, Y. H., Sun, Y., Knight, A. L., McLean, P. J., Caldwell, G. A., Sidransky, E., Grabowski, G. A. and Krainc, D.** (2011). Gaucher disease glucocerebrosidase and alpha-synuclein form a bidirectional pathogenic loop in synucleinopathies. *Cell* **146**, 37-52.
- McEachern, K. A., Nietupski, J. B., Chuang, W. L., Armentano, D., Johnson, J., Hutto, E., Grabowski, G. A., Cheng, S. H. and Marshall, J.** (2006). AAV8-mediated expression of glucocerebrosidase ameliorates the storage pathology in the visceral organs of a mouse model of Gaucher disease. *J. Gene Med.* **8**, 719-729.
- McEachern, K. A., Fung, J., Komarnitsky, S., Siegel, C. S., Chuang, W. L., Hutto, E., Shayman, J. A., Grabowski, G. A., Aerts, J. M., Cheng, S. H. et al.** (2007). A specific

- and potent inhibitor of glucosylceramide synthase for substrate inhibition therapy of Gaucher disease. *Mol. Genet. Metab.* **91**, 259-267.
- Mistry, P. K. and Zimran, A.** (2007). Type 1 Gaucher disease – clinical features. In *Gaucher Disease* (ed. A. H. Futerman and A. Zimran), pp. 155-173. Taylor and Francis Group: Boca Raton, FL.
- Mistry, P. K., Liu, J., Yang, M., Nottoli, T., McGrath, J., Jain, D., Zhang, K., Keutzer, J., Chuang, W. L., Mehal, W. Z. et al.** (2010). Glucocerebrosidase gene-deficient mouse recapitulates Gaucher disease displaying cellular and molecular dysregulation beyond the macrophage. *Proc. Natl. Acad. Sci. USA* **107**, 19473-19478.
- Mizukami, H., Mi, Y., Wada, R., Kono, M., Yamashita, T., Liu, Y., Werth, N., Sandhoff, R., Sandhoff, K. and Proia, R. L.** (2002). Systemic inflammation in glucocerebrosidase-deficient mice with minimal glucosylceramide storage. *J. Clin. Invest.* **109**, 1215-1221.
- Mu, T. W., Fowler, D. M. and Kelly, J. W.** (2008). Partial restoration of mutant enzyme homeostasis in three distinct lysosomal storage disease cell lines by altering calcium homeostasis. *PLoS Biol.* **6**, e26.
- O'Neill, R. R., Tokoro, T., Kozak, C. A. and Brady, R. O.** (1989). Comparison of the chromosomal localization of murine and human glucocerebrosidase genes and of the deduced amino acid sequences. *Proc. Natl. Acad. Sci. USA* **86**, 5049-5053.
- Rigat, B. and Mahuran, D.** (2009). Diltiazem, a L-type Ca(2+) channel blocker, also acts as a pharmacological chaperone in Gaucher patient cells. *Mol. Genet. Metab.* **96**, 225-232.
- Sardi, S. P., Clarke, J., Kinnecom, C., Tamsett, T. J., Li, L., Stanek, L. M., Passini, M. A., Grabowski, G. A., Schlossmacher, M. G., Sidman, R. L. et al.** (2011). CNS expression of glucocerebrosidase corrects [alpha]-synuclein pathology and memory in a mouse model of Gaucher-related synucleinopathy. *Proc. Natl. Acad. Sci. USA* **108**, 12101-12106.
- Schiffmann, R. and Vellodi, A.** (2007). Neuronopathic Gaucher disease. In *Gaucher Disease* (ed. A. H. Futerman and A. Zimran), pp. 175-196. Taylor and Francis Group: Boca Raton, FL.
- Shachar, T., Bianco, C. L., Recchia, A., Wiessner, C., Raas-Rothschild, A. and Futerman, A. H.** (2011). Lysosomal storage disorders and Parkinson's disease: beyond Gaucher disease. *Mov. Disord.* **26**, 1593-1604.
- Sidransky, E.** (2004). Gaucher disease: complexity in a "simple" disorder. *Mol. Genet. Metab.* **83**, 6-15.
- Sidransky, E., Sherer, D. M. and Ginns, E. I.** (1992). Gaucher disease in the neonate: a distinct Gaucher phenotype is analogous to a mouse model created by targeted disruption of the glucocerebrosidase gene. *Pediatr. Res.* **32**, 494-498.
- Sidransky, E., Nalls, M. A., Aasly, J. O., Aharon-Peretz, J., Annesi, G., Barbosa, E. R., Bar-Shira, A., Berg, D., Bras, J., Brice, A. et al.** (2009). Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N. Engl. J. Med.* **361**, 1651-1661.
- Sinclair, G. B., Jevon, G., Colobong, K. E., Randall, D. R., Choy, F. Y. and Clarke, L. A.** (2007). Generation of a conditional knockout of murine glucocerebrosidase: utility for the study of Gaucher disease. *Mol. Genet. Metab.* **90**, 148-156.
- Stephens, M. C., Bernatsky, A., Burachinsky, V., Legler, G. and Kanfer, J. N.** (1978). The Gaucher mouse: differential action of conduritol B epoxide and reversibility of its effects. *J. Neurochem.* **30**, 1023-1027.
- Sun, Y. and Grabowski, G. A.** (2010). Impaired autophagosomes and lysosomes in neuronopathic Gaucher disease. *Autophagy* **6**, 648-649.
- Sun, Y., Qi, X., Witte, D. P., Ponce, E., Kondoh, K., Quinn, B. and Grabowski, G. A.** (2002). Prosaposin: threshold rescue and analysis of the "neuritogenic" region in transgenic mice. *Mol. Genet. Metab.* **76**, 271-286.
- Sun, Y., Quinn, B., Witte, D. P. and Grabowski, G. A.** (2005). Gaucher disease mouse models: point mutations at the acid beta-glucosidase locus combined with low-level prosaposin expression lead to disease variants. *J. Lipid Res.* **46**, 2102-2113.
- Sun, Y., Quinn, B., Xu, Y. H., Leonova, T., Witte, D. P. and Grabowski, G. A.** (2006). Conditional expression of human acid beta-glucosidase improves the visceral phenotype in a Gaucher disease mouse model. *J. Lipid Res.* **47**, 2161-2170.
- Sun, Y., Liou, B., Quinn, B., Ran, H., Xu, Y. H. and Grabowski, G. A.** (2009). In vivo and ex vivo evaluation of L-type calcium channel blockers on acid beta-glucosidase in Gaucher disease mouse models. *PLoS ONE* **4**, e7320.
- Sun, Y., Ran, H., Zamzow, M., Kitatani, K., Skelton, M. R., Williams, M. T., Vorhees, C. V., Witte, D. P., Hannun, Y. A. and Grabowski, G. A.** (2010a). Specific saposin C deficiency: CNS impairment and acid beta-glucosidase effects in the mouse. *Hum. Mol. Genet.* **19**, 634-647.
- Sun, Y., Liou, B., Ran, H., Skelton, M. R., Williams, M. T., Vorhees, C. V., Kitatani, K., Hannun, Y. A., Witte, D. P., Xu, Y. H. et al.** (2010b). Neuronopathic Gaucher disease in the mouse: viable combined selective saposin C deficiency and mutant glucocerebrosidase (V394L) mice with glucosylsphingosine and glucosylceramide accumulation and progressive neurological deficits. *Hum. Mol. Genet.* **19**, 1088-1097.
- Sun, Y., Ran, H., Liou, B., Quinn, B., Zamzow, M., Zhang, W., Bielawski, J., Kitatani, K., Setchell, K. D., Hannun, Y. A. et al.** (2011). Isofagomine in vivo effects in a neuronopathic Gaucher disease mouse. *PLoS ONE* **6**, e19037.
- Tybulewicz, V. L., Tremblay, M. L., LaMarca, B. E., Willemsen, R., Stubblefield, B. K., Winfield, S., Zablocka, B., Sidransky, E., Martin, B. M., Huang, S. P. et al.** (1992). Animal model of Gaucher's disease from targeted disruption of the mouse glucocerebrosidase gene. *Nature* **357**, 407-410.
- Van De Water, N. S., Jolly, R. D. and Farrow, B. R.** (1979). Canine Gaucher disease-the enzymic defect. *Aust. J. Exp. Biol. Med. Sci.* **57**, 551-554.
- Vellodi, A., Tylki-Szymanska, A., Davies, E. H., Kolodny, E., Bemb, B., Collin-Histed, T., Mengel, E., Erikson, A. and Schiffmann, R.** (2009). Management of neuronopathic Gaucher disease: revised recommendations. *J. Inher. Metab. Dis.* **32**, 660-664.
- Vitner, E. B., Platt, F. M. and Futerman, A. H.** (2010a). Common and uncommon pathogenic cascades in lysosomal storage diseases. *J. Biol. Chem.* **285**, 20423-20427.
- Vitner, E. B., Dekel, H., Zigdon, H., Shachar, T., Farfel-Becker, T., Eilam, R., Karlsson, S. and Futerman, A. H.** (2010b). Altered expression and distribution of cathepsins in neuronopathic forms of Gaucher disease and in other sphingolipidoses. *Hum. Mol. Genet.* **19**, 3583-3590.
- Willemsen, R., Tybulewicz, V., Sidransky, E., Eliason, W. K., Martin, B. M., LaMarca, M. E., Reuser, A. J., Tremblay, M., Westphal, H., Mulligan, R. C. et al.** (1995). A biochemical and ultrastructural evaluation of the type 2 Gaucher mouse. *Mol. Chem. Neuropathol.* **24**, 179-192.
- Wong, K., Sidransky, E., Verma, A., Mixon, T., Sandberg, G. D., Wakefield, L. K., Morrison, A., Lwin, A., Colegial, C., Allman, J. M. et al.** (2004). Neuropathology provides clues to the pathophysiology of Gaucher disease. *Mol. Genet. Metab.* **82**, 192-207.
- Xu, Y. H., Quinn, B., Witte, D. and Grabowski, G. A.** (2003). Viable mouse models of acid beta-glucosidase deficiency: the defect in Gaucher disease. *Am. J. Pathol.* **163**, 2093-2101.
- Xu, Y. H., Reboulet, R., Quinn, B., Huelsken, J., Witte, D. and Grabowski, G. A.** (2008). Dependence of reversibility and progression of mouse neuronopathic Gaucher disease on acid beta-glucosidase residual activity levels. *Mol. Genet. Metab.* **94**, 190-203.
- Xu, Y. H., Sun, Y., Barnes, S. and Grabowski, G. A.** (2010). Comparative therapeutic effects of velaglucerase alfa and imiglucerase in a Gaucher disease mouse model. *PLoS ONE* **5**, e10750.
- Xu, Y. H., Sun, Y., Ran, H., Quinn, B., Witte, D. and Grabowski, G. A.** (2011a). Accumulation and distribution of alpha-synuclein and ubiquitin in the CNS of Gaucher disease mouse models. *Mol. Genet. Metab.* **102**, 436-447.
- Xu, Y. H., Jia, L., Quinn, B., Zamzow, M., Stringer, K., Aronow, B., Sun, Y., Zhang, W., Setchell, K. D. and Grabowski, G. A.** (2011b). Global gene expression profile progression in Gaucher disease mouse models. *BMC Genomics* **12**, 20.
- Yoneshige, A., Suzuki, K. and Matsuda, J.** (2010). A mutation in the saposin C domain of the sphingolipid activator protein (Prosaposin) gene causes neurodegenerative disease in mice. *J. Neurosci. Res.* **88**, 2118-2134.