Animal models for Gaucher disease research

Tamar Farfel-Becker¹,*, Einat B. Vitner¹,* and Anthony H. Futerman¹,‡

Introduction

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.

Gaucher disease (GD), the most common lysosomal storage disorder (LSD), is caused by the defective activity of glucocerebrosidase (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 absence of the lysosomal hydrolase glucocerebrosidase, 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 (type 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 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.

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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 defect in the skin (Holleran et al., 1994); this established a link between GlcCer accumulation and skin abnormalities 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). Nevertheless, these mice have been used to study the efficacy of a chemical chaperone as a treatment for GD (Khanha 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 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.

**RecNciI and L444P/L444P mice**

Mice carrying the recombinant allele traditionally known as the ‘RecNciI’ 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 RecNciI mutation developed more severe disease than those that were homozygous for L444P: RecNciI 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; RecNciI 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 RecNciI 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 GlcCerase synthase (Uggs) (Mizukami et al., 2002), the protein that is responsible for the synthesis of GlcCer, generating a Gba−/−,Uggs+/− mouse. A series of intercrosses of Gba−/−,Uggs+/− mice gave rise to adult mice that were L444P/L444P for the gene encoding GlcCerase and wild type for the Uggs gene; these are referred to as Gba−/−,Uggs+/−,L444P/+ 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, GlcCerase 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 (Khanha 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).

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### Animal models of Gaucher disease

**Commentary**

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

Fig. 1. A timeline of the generation of mouse models of GD. For further details, see text.
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.
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 oestroblast 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<sup>bik/bik</sup> mice were then bred with keratin-14-Cre transgenic mice (to create K14-lnl/lnl 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-lnl/lnl 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-lnl/lnl mice developed rapidly-progressing neurological disease, leading to continuous seizures and paralysis at 2 weeks of age, which was associated with neuronal loss and microgliosis 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 <i>loxP</i> 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-lnl/lnl 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-lnl/lnl 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 (Vittner 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-lnl/lnl 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 it 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


COMMENTARY

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