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Human Genome & Diseases: Review

Therapeutic strategies to ameliorate lysosomal storage disorders – a focus on Gaucher disease

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Abstract. The lysosomal storage disorders encompass more than 40 distinct diseases, most of which are caused by the deficient activity of a lysosomal hydrolase leading to the progressive, intralysosomal accumulation of substrates such as sphingolipids, mucopolysaccharides, and oligosaccharides. Here, we primarily focus on Gaucher disease, one of the most prevalent lysosomal storage disorders, which is caused by an impaired activity of glucocerebrosidase, resulting in the accumulation of the glycosphingolipid glucosylceramide in the lysosomes. Enzyme replacement and substrate reduction therapies have proven effective for Gaucher disease cases without central nervous system involvement. We discuss the promise of chemical chaperone therapy to complement established therapeutic strategies for Gaucher disease. Chemical chaperones are small molecules that bind to the active site of glucocerebrosidase variants stabilizing their three-dimensional structure in the endoplasmic reticulum, likely preventing their endoplasmic reticulum-associated degradation and allowing their proper trafficking to the lysosome where they can degrade accumulated substrate to effectively ameliorate Gaucher disease.

Keywords. Chemical chaperone therapy, enzyme replacement therapy, gene therapy, glucosylceramide, glucocerebrosidase, lysosomal hydrolase, protein folding, substrate reduction therapy.

Introduction

Glycosphingolipids are essential cell membrane components composed of a polysaccharide chain of varying length (≈1–40 residues) linked to ceramide by a glycosidic bond. Lysosomes contain at least 50–60 soluble hydrolases and several integral membrane proteins that degrade these types of substrates. Glycosphingolipids are sequentially degraded, releasing carbohydrates from the nonreducing terminal residue, ultimately generating ceramide and monosaccharides that are recycled in the cytoplasm. Mutations in genes that encode proteins central to this degradation pathway result in a metabolic (catabolism) block and, as a consequence, the storage of substrate in the lysosomes, leading to a lysosomal storage disorder (Fig. 1) [1, 2].

To date, more than 40 distinct lysosomal storage disorders have been described, most of which are due to the deficiency of a single lysosomal enzyme or essential cofactor that is required for the lysosome to carry out its normal degradative role [3]. For example, mucopolysaccharidosis II (MPS II, Hunter syndrome) results from a deficiency of the enzyme α-L-iduronate-2-sulfatase required for the degradation of mucopolysaccharides; Gaucher disease results from a deficiency in glucocerebrosidase (GC) involved in the degradation of glucosylceramide; and Niemann-Pick disease types A and B result from a deficiency in sphingomyelinase [1, 2]. Thus, a deficiency in any of these or other lysosomal proteins will result in the intralysosomal accumulation of undegraded metabolites and eventually lead to clinical symptoms associated with lysosomal storage disorders.

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Depending on the nature of the metabolic error (Fig. 1), the lysosomal storage disorders can be grouped into distinct classes, some of which are discussed below [1, 2]. The sphingolipidoses subgroup represents the most prevalent lysosomal storage disorders in which a defective activity of a lysosomal hydrolase results in the inability to degrade glycosphingolipids. The sphingolipidoses include Fabry disease, Gaucher disease, Niemann-Pick disease type A and B, and gangliosidoses such as Tay-Sachs and Sandhoff disease (Fig. 1). The mucopolysaccharidoses result from defects in lysosomal hydrolases such as the exoglycases or sulfatases, leading to the accumulation of glycosaminoglycans in the lysosome, including dermatan sulfate and heparan sulfate in case of MPS I (Hunter disease), and dermatan sulfate in MPS VI (Maroteaux-Lamy syndrome). The oligosaccharidoses and glycoproteinoses arise when a defect occurs in the production of enzymes (e.g., α-fucosidase and α-mannosidase) normally involved in the sequential cleavage of carbohydrate residues from glycoproteins. In addition, some

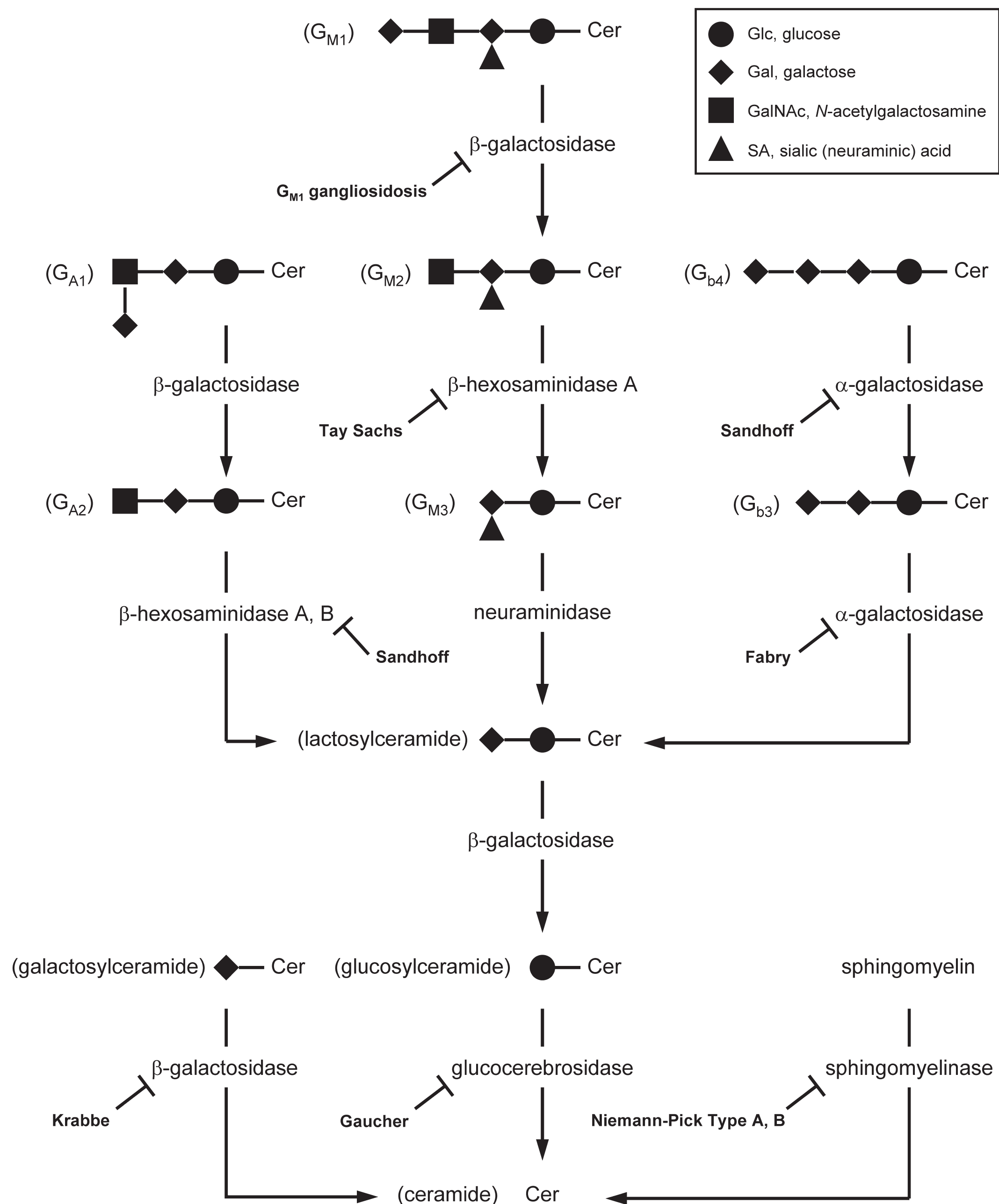

Figure 1. Degradation pathway for glycosphingolipids implicated in selected storage diseases. The glycosphingolipids are catabolized by sequential hydrolysis of terminal carbohydrate residues. Enzymatic blocks lead to storage of the indicated substrates and the respective lysosomal storage disorders indicated in bold.

lysosomal storage disorders have been described that are caused by defects in integral lysosomal membrane proteins. These include mucolipidosis, and Niemann-Pick disease type C, characterized by the accumulation of lipids and mucopolysaccharides, and cholesterol and sphingolipids, respectively.

While there are no two diseases with exactly the same pathology, the lysosomal storage disorders have some biochemical and clinical characteristics in common. In general, clinical symptoms are progressive over time, and include a range of somatic changes such as a characteristic facial appearance, coarse hair, enlarged liver and spleen, bone and skeletal changes, short stature, cloudy corneas, and respiratory or cardiac problems [3]. Many lysosomal storage disorders also lead to neurodegenerative changes as substrates accumulate in the brain. Patients affected by the Maroteaux-Lamy syndrome or Gaucher disease type 1 experience somatic changes only, while in other disorders, such as Hurler syndrome, patients may suffer from both somatic problems and brain dysfunction [3]. Individual lysosomal storage disorders are relatively rare, with an overall birth incidence between 1:5000 and 1:10,000 and unusually high incidences in some populations because of possible genetic drift and founder effects [4–6].

Most lysosomal storage disorders display a remarkable clinical heterogeneity – the same mutated gene can often cause a range of phenotypes varying from mild late-onset disease accompanying a normal lifespan to acute disease leading to infantile death. Residual enzyme activity has been identified as one of the factors influencing the clinical outcome of disease. The lack of any residual activity is frequently associated with severe pathology, whereas even small amounts of residual enzymatic activity (e.g., 5%) can substantially slow the clinical onset of disease. However, several cases with a relatively high residual activity were associated with significant disease, suggesting that there is no strict correlation between residual enzyme activity and disease phenotype. Thus, it would be inaccurate to predict the disease phenotype of a given patient based on the genotype, because patients carrying the same mutant alleles may show a different phenotypic variability due to modifying genes and epigenetic factors [7, 8] that influence the residual enzyme activity, and as a consequence the disease phenotype. Monozygotic twins have been reported with only one symptomatic twin, implying that nonheritable factors may also influence disease expression in genetically predisposed individuals [9]. While in most cases, inherited deficiencies of lysosomal hydrolase activity are associated with clinical disease, individuals have been reported who, despite having greatly reduced enzyme activity levels, remain healthy. Instances of such ‘pseudo-deficiencies’ have been reported to occur with several lysosomal hydrolases including arylsulfatase A, β-hexosaminidase A, galactocerebrosidase β-galactosidase, α-galactosidase A, α-L-iduronidase, β-glucuronidase, α-glucosidase, and α-L-fucosidase [10].

The phenomenon of pseudo-deficiency demonstrates that a residual lysosomal enzyme activity may be sufficient to overcome pathology. For example, a lack of the β subunit of β-hexosaminidase A associated with Sandhoff disease in its acute form leads to early death. Chronic adult forms occur with 3–6% of normal residual enzyme activity levels, while completely unaffected individuals have been identified with only 9–10% of normal levels [11]. Conzelmann and Sandhoff have proposed that 5–10% of normal β-hexosaminidase A levels represents a critical threshold for the development of disease symptoms [12]. Therefore, slight increases in the activity of mutant lysosomal enzymes may be clinically useful for ameliorating disease.

Obviously, lysosomal storage disorders represent a significant health problem. The study of their cellular basis may ultimately lead to a common therapeutic strategy to ameliorate these inherited diseases. This review focuses on therapeutic strategies for one of the most prevalent lysosomal storage disorders, Gaucher disease. We will focus on the promise of using small-molecule chemical chaperones to ameliorate Gaucher disease and related lysosomal storage disorders.

Gaucher disease

Gaucher disease was first described in 1882 by the French medical student, Phillipe C. E. Gaucher. He noticed the presence of large, unusual cells in a 32-year-old female subject with an enlarged spleen. The storage material in these cells was identified as glucosylceramide (glucocerebroside) in 1934 [13] (Fig. 2). More than 30 years later, Brady and colleagues demonstrated that the primary defect in these cells was a deficient activity of the lysosomal hydrolase GC (acid β-glucosidase, glucosylceramidase, D-glucosyl-*N*-acylsphingosine glucohydrolase) [14]. Despite many years of research to decipher the biochemical and molecular genetic aspects of Gaucher disease, reports explaining how the accumulation of glucosylceramide in lysosomes confers cellular pathol-

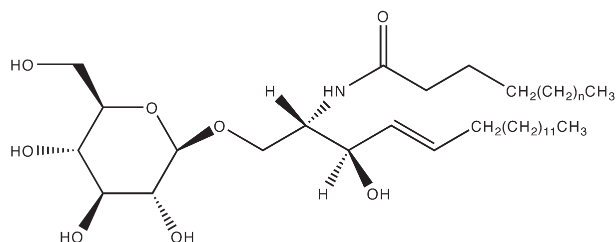

Figure 2. Representation of the chemical structure of D-glucosylceramide, the substrate stored in Gaucher disease. n = 10–18.

ogy and Gaucher disease-associated symptoms remain scarce [15]. Changes in phospholipid metabolism in neuronal models of Gaucher disease [16], and the correlation between Ca^{2+} release from brain microsomes obtained from post-mortem Gaucher disease patients and the level of glucosylceramide accumulation [17] further add to our understanding as to how the lysosomal glucosylceramide accumulation leads to Gaucher disease symptoms.

To date, approximately 200 mutations in the GC gene have been reported to be associated with Gaucher disease [18]. Those GC variants exhibit a decreased GC intra-lysosomal activity leading to the accumulation of undegraded glucosylceramide in the lysosomes of monocyte-macrophage cells. This results in a broad range of symptoms that may include hepatomegaly, splenomegaly, anemia and thrombocytopenia, bone lesions, and central nervous system (CNS) involvement. Patients without CNS symptoms are classified as type 1, while those exhibiting CNS symptoms are classified as either type 2 (acute infantile) or type 3 (juvenile or early adult onset).

Gaucher disease occurs with a frequency between 1:40,000 and 1:60,000 in the general population, and between 1:500 and 1:1000 among the Ashkenazi Jewish population [19]. The majority of the patients (95%) have type 1 disease [20]. More evidence is becoming available that suggests a correlation between Gaucher disease and the development of parkinsonism [21].

While many disease-causing Gaucher alleles have been identified, the most frequently encountered mutations include N370S (1226G), 84GG, L444P (1448C), IVS2+1, D409H (1342C), R496H (1604A), and F213I (754T) [22]. The allele distributions are population dependent. Sidransky and colleagues genotyped 54 type 1 Ashkenazi Jewish subjects and 64 type 1 non-Jewish subjects [23]. Their results roughly support the allele distributions previously compiled by Grabowski and Horowitz [22], although improved screening methods have allowed the identification of numerous Gaucher disease-associated alleles that previous screening methodologies would have classified as only having the point mutation L444P. Remarkably, over 75% of the alleles among the Ashkenazi Jewish subjects were N370S (Fig. 3). The N370S allele was also the most prevalent allele in the non-Jewish subjects, albeit at a lower frequency than in the Ashkenazi Jewish population. The L444P allele (37.5%) occurred at a much higher frequency among non-Jewish subjects (Fig. 3) [23]. A novel GC variant, L371V, has recently been discovered in a Lebanese family causing symptoms that were more severe than those associated with the common N370S mutation [24]. It is worthwhile to note that much more is known about mutations associated with Gaucher disease patients of European descent than there is of patients originating from other parts of the world.

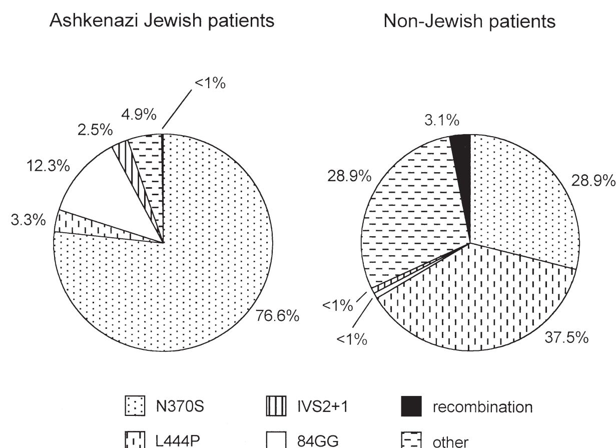

Figure 3. The distribution of five common mutant alleles among Ashkenazi Jewish (108 alleles) and non-Jewish (128 alleles) patients with type 1 Gaucher disease. Figure adapted from Koprivica et al. [23].

Because there is significant genotypic heterogeneity among clinically similar patients and phenotypic diversity among patients with the same mutations, attempts to draw genotype-phenotype correlations have proven to be difficult. For example, Beutler and colleagues [25] have estimated that only 30% of Ashkenazi Jewish people who are homozygous for the N370S mutation develop sufficient pathology to be ascertained medically. The frequency of common Gaucher disease alleles is summarized in Table 1.

The data suggest that the presence of the N370S allele leads to a nonneuronopathic phenotype, even in compound

Table 1. Distribution of Gaucher disease alleles among the three disease types.

		N370S		L444P		84GG		IVS2+1		recombination		other	
	Total[1]	#	%	#[2]	%	#	%	#	%	#	%	#	%
type 1[3]	236	121	51.3	22	9.3	15	6.4	5	2.1	17	7.2	56	23.7
type 2[4]	62	–	–	12	19.4	–	–	2	3.2	15	24.2	36	58.1
type 3[3]	48	–	–	20	41.7	2	4.2	2	4.2	5	10.4	19	39.6

[1] This row represents the total number of alleles (total), the respective number of alleles (#), and the percentage (%).
[2] Does not include L444P mutations that are part of *recA-recF* mutations. When these are included, the detected L444P allele frequency is 25 (41%), which is similar to that estimated by Grabowski and Horowitz [22].
[3] Data adapted from Koprivica et al. [23].
[4] Data adapted from Stone et al. [26].

Table 2. Age of onset of symptoms in Ashkenazi Jewish patients with the three most common genotypes.

Genotype	Age of onset (years)	
	mean	range
N370S/N370S	29.8	3–73
N370S/84GG	10.7	1–59
N370S/L444P	15.4	2–36

Adapted from Zhao and Grabowski [27].

heterozygotes. The nature of the second allele may have some impact on the age of onset of symptoms (Table 2). Patients who are homozygous for N370S tend to have a later age of onset than heterozygous individuals who have N370S and a different mutant allele, although the range is extremely broad. The phenotypic differences between N370S/N370S patients, N370S/L444P patients, and N370S/84GG patients highlight that small changes in levels of GC activity may have a large impact on the disease pathology. The wide ranges in age of onset in all three common genotypes reflects the phenotypic heterogeneity of the disease. Interestingly, Ron and Horowitz [28] reported a significant correlation between endoglycosidase-H sensitivity, endoplasmic reticulum (ER) retention and degradation of GC, and disease severity. Patients with the same genotype may exhibit differences in disease severity because of individual differences in the factors that determine the degradation of proteins within the ER [28].

Gaucher disease is an attractive target for the development of pharmaceutical interventions due to its relatively high occurrence, the prevalence of a relatively small number of alleles, and the lack of CNS involvement in the majority of patients (Gaucher disease type 1). Insight gained from biochemical and cell biological characterization and the development of therapeutics for this disease should facilitate the development of treatments for other lysosomal storage disorders.

Enzyme replacement therapy

In 1964, Noble Prize winner de Duve remarked that, ‘Any substance that is taken up intracellularly by an endocytic process is likely to end up within lysosomes. This obviously opens up many possibilities for interaction, including replacement therapy’ [29]. This suggested that the endogenous activity of a defective lysosomal hydrolase may be increased by the administration of exogenous enzyme and, in fact, led to the development of the first therapeutic strategy, namely enzyme replacement therapy (ERT), for the treatment of a lysosomal storage disorder.

ERT has been developed to treat several lysosomal storage disorders, including Gaucher disease, and has recently been approved for the treatment of Fabry disease [30], MPS I [31], and MPS VI [32] (Table 3). Clinical trials are ongoing evaluating enzymes to treat Pompe disease [33] and MPS II [34], and preclinical studies will be performed in the near future for several other disorders, including metachromatic leukodystrophy [35], α-mannosidosis [36], and Niemann-Pick disease type B [37]. Responses to ERT in Fabry disease, Pompe disease, Hurler disease, and Maroteaux-Lamy syndrome have generally been encouraging, although variations in the extent of benefit have been observed. Improving the delivery of therapeutic enzymes to affected tissues such as the cardiac muscle and kidney (Fabry disease), skeletal muscle (Pompe disease), and to joint tissues (MPS disorders)

Table 3. Enzyme replacement therapy for lysosomal storage disorders.

Disease	Enzyme replaced	Company	Status
Gaucher, type 1 and type 3	glucocerebrosidase	Genzyme	approved EU/US (1991)
Fabry	α-galactosidase A	Genzyme	approved EU (2001) approved US (2003)
		Transkaryotic Therapies	approved EU (2001)
MPS I (Hurler)	α-L-iduronidase	BioMarin Pharmaceutical/Genzyme	approved EU/US (2003)
MPS IV (Maroteaux-Lamy)	arylsulfatase B	BioMarin Pharmaceutical	approved US (2005)
Pompe	α-glucosidase	Genzyme	phase III clinical trial
MPS II (Hunter)	α-L-iduronate sulfatase	Transkaryotic Therapies	phase III clinical trial
Niemann-Pick B	acid sphingomylinase	Genzyme	preclinical
Metachromatic leukodystrophy	arylsulfatase A	Zymenex	preclinical
α-Mannosidosis	1183α-mannosidase	Zymenex	preclinical

would certainly enhance the clinical response [38]. Furthermore, an all-encompassing concern in developing ERT for lysosomal storage diseases is to devise methods to ameliorate the damage to the central and peripheral nervous systems that occurs in specific phenotypes of these disorders [38].

Attempts to develop ERT for Gaucher disease were initially pursued using GC purified from human placenta. The results were not impressive [39, 40], likely due to inefficient localization of the exogenous GC to the enzymatically deficient macrophages. Identification of a mannose-specific receptor on macrophages made it possible to specifically target this cell type [41, 42]. Modification of placental GC by sequentially removing sialic acid, *β*-galactose, and *β*-*N*-acetylgalactosamine resulted in a 'mannose-terminated' form of the enzyme that is indeed recognized by mannose receptors present on the surface of macrophages. This results in endocytosis and delivery to the lysosome where it supplements the defective enzyme [43]. Weekly intravenous infusions of the macrophage-targeted preparation of human placental GC in a male subject with type 1 Gaucher disease led to substantial clinical improvement [44].

The first clinical trial with commercially produced mannose-enriched placental GC (Ceredase, Genzyme Corporation, Boston, Mass.) was performed with 12 type 1 Gaucher subjects [45]. The subjects received 60 IU per kilogram of body weight every 2 weeks for 9–12 months. Two of the patients received infusions weekly during part of the trial because they had a clinically aggressive disease. All of the subjects displayed a marked improvement in organomegaly and corrections of hematological abnormalities. The hemoglobin concentration increased in all 12 subjects, and the platelet count in 7. Splenic and hepatic volumes decreased in all patients within 6 months of treatment. While early signs of skeletal improvements were seen in three subjects, hematologic and visceral responses to enzyme replacement appeared to develop more rapidly than the skeletal response.

A major contribution to the field was the cloning of the gene encoding GC [46] which paved the way to develop a recombinant human enzyme produced in Chinese hamster ovary cells (Cerezyme, Genzyme Corporation). Studies have demonstrated that the mannose-enriched recombinant enzyme has a similar therapeutic effect as GC purified from human placenta [47]. To date, more than 3000 type 1 Gaucher disease patients have received ERT [48, 49], including an Australian population [50], and this success has definitely encouraged the development of recombinant enzymes for the treatment of other lysosomal storage diseases.

While ERT has been shown to be a safe and effective treatment for type 1 Gaucher disease as described above, this therapy has some limitations [51]. Most significant is the inability of an ≈60-kDa glycoprotein to cross the blood-brain barrier. As a consequence, the amelioration of the CNS involvement of chronic neuronopathic disease (type 2 and 3 Gaucher disease) remains difficult if not impossible [52]. The burden of receiving intravenous infusions every two weeks also limits the availability of ERT to all patients who need it. In addition, the high cost of Cerezyme represents an economic burden. For example, the dose used in the clinical trial mentioned above costs US $ 16,800 every 2 weeks for a 70-kg patient [53], and by extension an annual cost of more than US $ 430,000 per patient. This extremely high annual cost per patient makes it unlikely that ERT can be utilized for the treatment of Gaucher disease patients. Furthermore, in contrast to most of the other pathological symptoms, ERT does not ameliorate Gaucher disease-related hematological symptoms including an increased mean cell volume, whole-blood viscosity, and reduced relative filtration rate observed in Gaucher disease patients with no functional spleen [54]. While at present there is no clear consensus about optimal dosing regimens during maintenance ERT, it is known that only a small fraction of the administered enzyme is able to reach bone marrow macrophages [55, 56]. Although some improvement of the skeletal complications associated with Gaucher disease type 1 during ERT has been reported [57], more recent reports indicate that complimentary therapeutic strategies are needed to restore existing bone abnormalities and/or prevent changes in the bone mineral density during ERT [58–60]. The latter may be achieved by the intake of biphosphonates such as pamidronate [61] or alendronate disodium [62] in parallel with ERT.

In summary, further optimization of dosing regimens, improving enzyme delivery to cells in target organs, as well as engineering a version of the enzyme which is not so rapidly cleared from the blood should help increase the effectiveness and reduce the high cost of ERT for Gaucher disease.

Substrate reduction therapy

Substrate reduction therapy (SRT) is based on the hypothesis that disease phenotype is governed by the ratio of residual lysosomal activity to substrate influx into the lysosome [63]. In other words, the pathological, intralysosomal accumulation of undegraded substrate will proceed in individuals with insufficient substrate-degrading enzyme activity. Since reducing substrate influx will reduce its accumulation, inhibition of substrate biosynthesis may improve the clinical course of these maladies. Complex glycosphingolipids are assembled by the stepwise addition of sugars to glucosylceramide in the lumen of the Golgi [64]. Glucosylceramide is synthesized by the enzyme glucosyltransferase on the cytosolic surface of the Golgi [65–68]. Thus, inhibition of the action of glu-

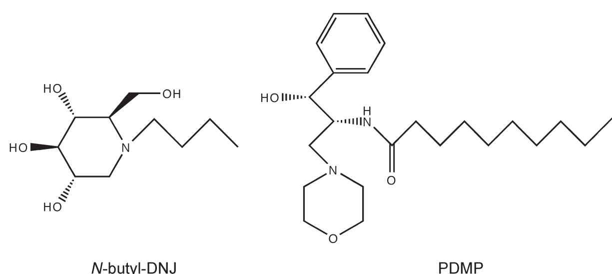

Figure 4. Representation of the chemical structures of the glucosyltransferase inhibitors *N*-butyl-deoxynojirimycin (*N*-butyl-DNJ) and 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) employed to reduce substrate biosynthesis.

cosyltransferase should reduce the formation of all glucosylceramide-based glycosphingolipids and this may be applicable to any of the diseases that are characterized by the lysosomal storage of glucosylceramide-based glycosphingolipids (Fig. 1). This concept was first proposed as a therapy for Gaucher disease by Radin and coworkers, although the lack of an appropriate animal model for Gaucher disease made it difficult to test the hypothesis [69]. Since a variety of glycolipids accumulate in Niemann-Pick disease type C cells, SRT may also be useful for correcting the abnormal lipid trafficking observed in B lymphocytes of Niemann-Pick disease type C patients [70]. Currently, SRT is in phase III clinical trials for Gaucher disease type 3, late-onset Tay-Sachs disease, and Niemann-Pick disease type C.

Two main classes of inhibitors of glucosyltransferase have been used in animal models of various lysosomal storage disorders. The first class of inhibitors were designed to be analogues of ceramide, the prototype inhibitor being 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP; Fig. 4) [71]. Analogues with improved selectivity and potency have been developed by exchanging the morpholine ring for a pyrrolidine ring and adding substituents to the phenyl ring [72]. Oral administration of a PDMP analogue to α-galactosidase A knockout mice, an animal model for Fabry disease, resulted in a considerable reduction in the globotriaosylceramide content (G_{b3}, Fig. 1) [73].

N-alkylated iminosugars, such as *N*-butyl-deoxynojirimycin (*N*-butyl-DNJ; Fig. 4), are also inhibitors of glycosyltransferase [74]. *N*-butyl-DNJ was studied in clinical trials as an inhibitor of HIV infection due to its potency as a glucosidase inhibitor [75]. While sufficient concentrations of *N*-butyl-DNJ for antiviral activity could not be achieved in humans, these trials established important medicinal safety information. Platt and colleagues [76] demonstrated that *N*-butyl-DNJ has the ability to inhibit glucosyltransferase, and thus the compound was further investigated in models of lysosomal storage disorders. Oral administration of *N*-butyl-DNJ to a Tay-Sachs murine model was found to reduce G_{M2} ganglioside storage in neurons, demonstrating that these compounds are able to cross the blood-brain barrier and thus may be effective for treating the CNS involvement in many lysosomal storage disorders [77]. Animal studies have shown that the galactose analogue of *N*-butyl-DNJ, *N*-butyl-deoxygalactonojirimycin, may have the same efficacy as *N*-butyl-DNJ, but does not cause gastrointestinal side effects [78].

The first clinical trial of SRT employed *N*-butyl-DNJ to treat 28 Gaucher disease type 1 patients [79]. Patients received 100 mg of *N*-butyl-DNJ (Zavesca, Actelion Pharmaceuticals, Allschwil, Switzerland) three times daily. Continual improvements in organomegaly and hematological abnormalities were noticed between 6 and 12 months of treatment, although the extent of the response is less than that generally seen with ERT. The therapeutic effect is dose dependent, and a lower dose of *N*-butyl-DNJ (50 mg three times daily) is far less effective [80].

Unlike Cerezyme, Zavesca administered orally appears to cross the blood-brain barrier. The long-term reduction in glycolipid levels may lead to unforeseen side effects, as the variety of essential roles that these macromolecules play in normal cell physiology is only partially understood [81, 82]. Nevertheless, although some toxic side effects have been reported by patients taking Zavesca [51], an extended clinical trial during which Gaucher disease type 1 patients were orally administered with a Zavesca dose of 100–300 mg (three times daily for 36 months) demonstrated that Zavesca was effective over time and tolerable for the patients during a time period of at least 3 years [83]. Statistically significant improvements were observed in organ volumes and hematological parameters. The diarrhea and weight loss reported initially decreased in magnitude and prevalence during the second and third year of the treatment [83]. However, due to the side effects reported during the first 6–12 months of the treatment, Zavesca has been approved in Europe and the United States only for use in patients with mild to moderate Gaucher disease type 1 for whom ERT is not a feasible option, although this may ultimately change.

Chemical chaperone therapy

Chemical chaperones are small molecules that bind to and stabilize misfolding-prone proteins in the ER, likely reducing their tendency to be degraded by the proteasome (Fig. 5). This appears to result in increased trafficking of variant proteins out of the ER through the Golgi and on to their destination environment. The increased folding and trafficking efficiency results in a concomitant increase in residual activity provided that the protein is stable in its destination environment. A number of nonspecific, low-molecular-weight compounds such as glycerol, dimethyl sulfoxide, trimethylamine *N*-oxide, and deuterated water

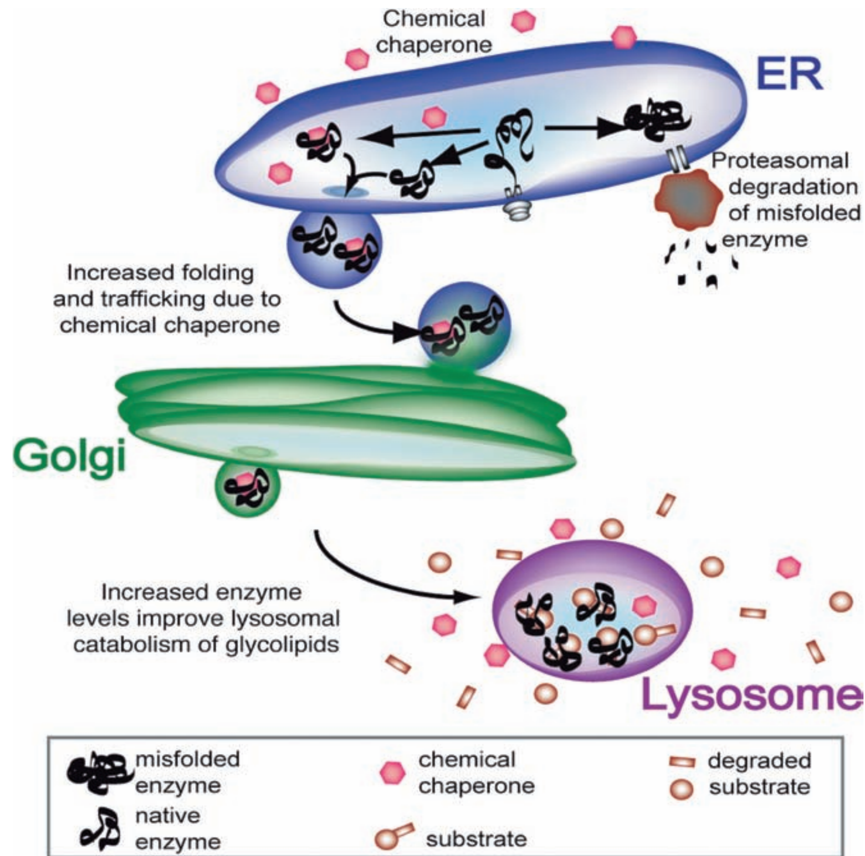

Figure 5. The proposed mechanism of chemical chaperoning. Lysosomal hydrolase folding commences in the endoplasmic reticulum (ER) at neutral pH. The folded enzyme is trafficked through increasingly acidic compartments to the lysosome. Enzyme that is deficient in folding in the ER is retained by chaperone binding and is targeted for degradation by the proteasome. Chemical chaperones stabilize the native state of the enzyme at neutral pH in the ER leading to increased trafficking to the lysosome. An enzyme that is unstable in the pH 7 environment of the ER may be stable and catalytically active in the acidic lysosomal environment.

have been shown to increase proper folding and trafficking of variant proteins when included in the culture medium at high (mM) concentrations [84]. These osmolytes function by destabilizing the unfolded states of all proteins, which renders the folded states 4 Kcal more stable owing to the protein solution environment. Compounds that bind selectively to the protein of interest (often at the active site), sometimes called pharmacological or chemical chaperones, are typically effective at much lower concentrations (nM to µM) and have the potential to be developed into disease-specific drugs [85] that can be used in chemical chaperone therapy (CCT).

Ishii and colleagues [86] have identified Fabry disease-associated α-galactosidase A variants (Q279E and R301Q) that display wild-type-like levels of activity when expressed in insect cells grown at room temperature. In contrast, their activity is much lower in cells grown at 37 °C. The apparent temperature sensitivity of these variants is suggestive of a deficiency in α-galactosidase A folding. The Q279E variant was found to have kinetic properties similar to wild-type α-galactosidase A [87], but this variant is thermolabile at neutral pH rendering it susceptible to misfolding in the environment of the ER and to ER-associated degradation mediated by the proteasome [86]. Galactose and reduced temperature were found to stabilize Q279E α-galactosidase A at neutral pH *in vitro* and to increase Q279E α-galactosidase A residual activity in tissue culture [86]. Galactose was thus identified as the first active site-directed chemical chaperone for a hydrolase implicated in a lysosomal storage disorder. Subsequent work identified several more α-galactosidase A variants in patient cell lines (e.g., A156V, L166V, G260A, G373S, R301Q and M296I) that are amenable to chemical chaperoning in the presence of 200 mM galactose. There is a subset of Fabry disease-associated mutations that appear not to be amenable to chemical chaperoning. These include the C142Y, E66Q/R112C, G328R, and N320K α-galactosidase A variants [88]. The crystal structure of C142Y α-galactosidase A illustrates that this mutation removes a required disulfide bond near the active site resulting in a catalytically inactive protein [89]. It is not clear why the E66Q/R112C, G328R, and N320K variants are not amenable to chemical chaperoning by galactose under the tested conditions. The authors con-

clude that many of the missense mutations in the α-galactosidase A gene allow expression of catalytically active proteins that are subjected to abnormal intracellular degradation [88]. Galactose binding protects the nascent enzyme from intracellular inactivation and degradation and leads to increased trafficking to the lysosome (Fig. 5). Galactose administration may be an effective therapy as investigated in a Fabry disease patient expressing the G328R α-galactosidase A variant. A 55-year-old male subject received galactose infusions (1 g per kilogram body weight) every other day and was followed for more than 2 years. The therapy was well tolerated and the clinical improvement was sufficient enough that cardiac transplantation was no longer required [90]. Such a positive clinical outcome supports the development of other nontoxic chemical chaperones for α-galactosidase A with higher efficacy and selectivity, although it must be recognized that this was a one-patient study.

Fan and coworkers have identified potent iminosugar inhibitors of α-galactosidase A, including 1-deoxygalactonojirimycin, that stabilize R301Q α-galactosidase A *in vivo*, and increase R301Q α-galactosidase A residual activity in tissue culture and in transgenic mice [91, 92]. Successful clearance of lysosomal G_{b3} storage and a near-normal lysosomal phenotype was also achieved in patient fibroblasts treated with 1-deoxygalactonojirimycin [93]. An active site-directed chemical chaperone for α-galactosidase A to treat Fabry disease developed by Amicus Therapeutics is currently in phase I clinical trials.

Recently, proof of concept for CCT has also been established in another lysosomal storage disease. Tropak and colleagues [94] have reported that growing adult Tay-Sachs fibroblasts in the presence of known inhibitors of β-hexosaminidase A raises the residual protein and activity levels of intralysosomal β-hexosaminidase A. They reveal a similar effect in fibroblasts from an adult Sandhoff patient, leading to the proposal that these hexosaminidase inhibitors function as chemical chaperones.

Matsuda and colleagues [95] have identified a potent iminosugar inhibitor of lysosomal β-galactosidase that restores mutant enzyme activity in G_{M1} gangliosidosis patient fibroblasts. Short-term oral administration of the inhibitor to a mouse model of juvenile G_{M1} gangliosidosis resulted in significant enhancement of the enzyme activity in the brain and other tissues. Immunohistochemical staining revealed a decrease in the amount of G_{M1} and G_{A1} stored in neuronal cells. These data demonstrated for the first time that CCT might be useful to successfully treat a CNS-targeted lysosomal storage disorder. The possibility that chemical chaperones can be designed to target specific glycosphingolipidoses extends the exciting repertoire of these novel therapeutics.

Taken together, CCT combines the specificity of an enzyme-directed approach (such as ERT) with the oral bioavailability and the potential to cross the blood-brain barrier of a small-molecule approach (such as SRT). In addition, chemical chaperones may be developed into a novel pharmacological treatment that is more convenient to use and better suited for long-term administration than current therapies, such as ERT and SRT. Moreover, these compounds could be useful for treating CNS-associated lysosomal storage disorders.

CCT for Gaucher disease

Previously, we have demonstrated that iminosugars such as *N*-(n-nonyl)deoxynojirimycin (*N*-nonyl-DNJ) added to Gaucher disease patient-derived fibroblasts significantly increased the activity of GC in a dose-dependent fashion, and showed that the potency of such small-molecule chemical chaperones to enhance the activity of the N370S GC variant was dependent on the nature of the iminosugar core and the length of the alkyl moiety [96]. Furthermore, we have investigated the generality of this approach by identifying several classes of iminosugar GC inhibitors that are able to alter the cellular localization of G202R GC and increase the cellular activity of N370S and G202R GC [97]. Noteworthy is the observation that several GC variants are retained in the ER and degraded by ER-associated degradation [28, 98]. Thus, chemical chaperones such as *N*-nonyl-DNJ may increase the cellular activity of G202R GC by binding to the active site of GC, stabilizing G202R GC in the ER preventing its degradation by the proteasome and enhancing its trafficking to the lysosome [97]. Although it remains to be proven, it appears that N370S and G202R GC are considerably less stable within the neutral pH environment of the ER than in the acidic environment of the lysosome. The presence of chemical chaperones may confer wild-type-like stability on those GC variants within the ER environment enhancing their trafficking to the lysosome – an environment that supports the N370S and G202R GC fold. In addition, *N*-butyl-DNJ (Zavesca) has recently been reported to exhibit a chaperone-like effect on GC variants carrying mutations in domain III (residues 76–381 and 416–430) and domain I (residues 1–27 and 383–414) [99]. While we have yet to identify a small molecule that can chaperone the L444P variant in patient-derived fibroblast cells, it appears that the location of this mutation in a noncatalytic domain of GC will likely necessitate the development of small molecules that bind to that domain of the enzyme in order to avoid ER-associated degradation.

Like SRT and in contrast to ERT, CCT relies on the endogenous activity of mutant enzyme. Chemical chaperoning will not be able to restore activity to cells with alleles such as 84GG that do not produce a protein product. Chaperoning enzymes that are catalytically inactive when folded will also not lead to increased residual activity by

this method, and it is unlikely that CCT can assist enzymes whose three-dimensional structures are unstable in the environment of the lysosome such as V394L, which is less stable at acidic pH than neutral pH [100]. Enzymes that are unable to bind the chaperone will not benefit from CCT. In addition, a mutation in a domain remote from the chemical chaperone-binding domain may be aided by small-molecule binding in another domain as long as the stabilities of the domains are thermodynamically linked. The amenability of a given mutation to chemical chaperoning probably will have to be determined empirically. Lastly, although *N*-alkylated DNJ analogues rapidly cross the plasma membrane in a fashion which is independent of the *N*-alkyl moiety [101], the more hydrophobic DNJ analogues such as *N*-nonyl-DNJ are retained in the gastrointestinal system for a longer time after oral administration to mice [102] which may exhibit cytotoxic effects caused by membrane perturbations [103].

To date, three GC variants have been shown to be amenable to chemical chaperoning. We have increased the activity of the highly prevalent N370S GC variant and the less common G202R GC variant by culturing patient fibroblasts with a variety of iminosugar compounds [97]. Lin and colleagues [104] have recently reported that application of *N*-octyl-*β*-valienamine to cells in tissue culture increases the activity of F213I GC. However, chemical chaperones that are effective with other GC variants do not increase the activity of L444P GC [97]. The crystal structure of GC provides some insight into the implications of these results [105]. All of the GC variants that are amenable to chemical chaperoning have mutations that are located in the active site domain, whereas the L444P mutation is located in the Ig-like domain of GC (Fig. 6). Stabilization of the active-site domain by inhibitors is apparently insufficient to stabilize the Ig domain harboring the L444P mutation, although it may be possible to use a small molecule that binds to the Ig-like domain to correct the folding defect exhibited by the L444P variant.

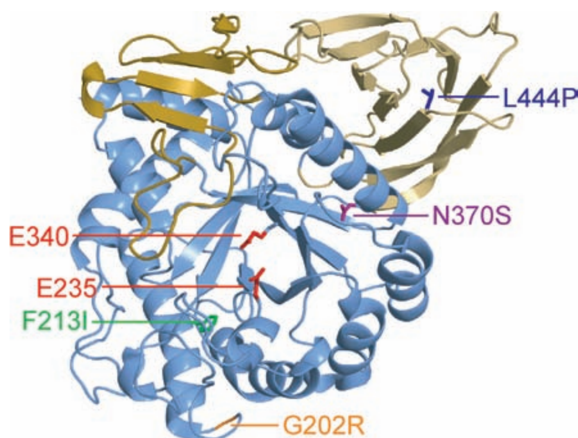

Figure 6. Locations of the GC mutations that have been tested for rescue by chemical chaperones. The catalytic nucleophiles, E235 and E340, are shown in red. Variants that are amenable to chemical chaperoning – G202R (orange), F213I (green), and N370S (purple) – are located in the same domain as the active site. L444P (blue) is located in the Ig-like domain remote from the active site and is not amenable to chaperoning by active site-targeted compounds.

Other therapeutic strategies for Gaucher disease

Although bone marrow transplantation seemed to be successful for nonneuronopathic Gaucher disease patients [106, 107], this approach is in general not considered as a feasible therapy for Gaucher disease [15] because of its high risk for mortality and the difficulties associated with finding a suitable donor. Initially thought to be of great promise for Gaucher disease patients, clinical trials applying hematopoietic stem cell-mediated gene therapy to ameliorate Gaucher disease type 1 were only successful in a minority of the patients [108], although it is important to realize that with continued improvements this therapy may become viable. Much progress has been made in the design of improved vectors including recombinant adeno-associated viral vectors [109] and HIV-1-based lentivirus vectors [110] that express human GC cDNA. Mice transplanted with lentivirus-transduced bone marrow-derived hematopoietic stem cells overexpressing human GC exhibited relatively high levels of GC activity in various tissues including bone marrow and spleen, but also the brain, 2–6 months after transplantation [111]. Although the suitability of the latter strategy to ameliorate Gaucher disease-associated symptoms in humans remains to be demonstrated, the results obtained in mice are encouraging.

Therapeutic outlook for Gaucher disease and other lysosomal storage diseases

A decade ago, treatment options for glycosphingolipid storage disorders were limited to ERT for Gaucher disease type 1. Recent advances have lead to the development of several mechanistically distinct therapeutic approaches to combat the symptoms caused by the intralysosomal accumulation of undegraded substrates.

ERT directly increases the lysosomal activity of deficient hydrolases by targeting recombinant enzyme to the lysosomes of deficient cells. While ERT has proven useful to treat many lysosomal storage disorders, targeting the exogenous enzyme to tissues of interest remains a challenge, especially in the brain.

Substrate reduction therapy inhibits the biosynthesis of glycosphingolipids on the cytoplasmic surface of the Golgi and thus reduces the formation of the various compounds stored in numerous lysosomal storage disorders.

This small molecule-based therapy is attractive because one compound can be used to treat multiple lysosomal storage disorders, including those with CNS involvement. The potential side effects of this therapeutic strategy will become better understood as the roles of these lipids in normal cell biology are elucidated and through clinical trials.

Currently, chemical chaperoning exhibits promise as a means of increasing the endogenous activity of several mutant lysosomal hydrolases. Active site-binding compounds stabilize these misfolding-prone enzymes in the ER enabling their folding and trafficking to the lysosome reducing the extent of ER-associated degradation. While these compounds have the potential to be developed into GC-selective, orally administered drugs that can cross the blood-brain barrier and ameliorate CNS symptoms, their application will be limited to the subset of variants that are prone to misfolding, but otherwise have enzymatic activity in their folded states. In most lysosomal storage disorders, the enzymes amenable to chemical chaperoning are the clinically most important ones.

It remains to be seen whether these distinct therapeutic approaches are complementary and may be used in conjunction with each other. It seems likely that SRT will be more effective if the lysosomal enzyme activity is elevated using ERT or chemical chaperones and vice versa. Importantly, small-molecule therapeutics have the potential to reach tissues that ERT has yet to effectively target, specifically brain tissue.

Significant progress has been made in developing Gaucher disease therapeutic strategies. Lessons learned from Gaucher disease have already yielded products that are being used to treat other lysosomal storage disorders. Improving ERT, optimizing SRT, and developing clinically optimal chemical chaperones will undoubtedly benefit patients with these rare, but devastating lysosomal storage disorders.

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