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Biochim Biophys Acta Gen Subj. Author manuscript; available in PMC 2021 January 01.

Published in final edited form as:

Biochim Biophys Acta Gen Subj. 2020 January ; 1864(1): 129437. doi:10.1016/j.bbagen.2019.129437.

Progress in the understanding and treatment of Fabry disease

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Abstract

Background: Fabry disease is caused by α-galactosidase A deficiency. Substrates of this lysosomal enzyme accumulate, resulting in cellular dysfunction. Patients experience neuropathic pain, kidney failure, heart disease, and strokes.

Scope of review: The clinical picture and molecular features of Fabry disease are described, along with updates on disease mechanisms, animal models, and therapies.

Major conclusions: How the accumulation of α-galactosidase A substrates, mainly glycosphingolipids, leads to organ damage is incompletely understood. Enzyme replacement and chaperone therapies are clinically available to patients, while substrate reduction, mRNA-based, and gene therapies are on the horizon. Animal models exist to optimize these therapies and elucidate disease mechanisms for novel treatments.

General significance: Recent newborn screening studies demonstrate that Fabry disease is the most common lysosomal storage disease. As many countries now include Fabry disease in their screening panels, the number of identified patients is expected to increase significantly. Better knowledge of disease pathogenesis is needed to improve treatment options.

Keywords

Fabry disease; lysosomal storage disease; glycosphingolipids; enzyme replacement therapy; chaperone therapy; rodent models

Introduction

Fabry disease (OMIM #301500) was first described in 1898 by two dermatologists, William Anderson [1] and Johannes Fabry [2]. Sixty-five years later, Sweeley and Klionsky discovered that patients with Fabry disease accumulate the glycosphingolipid, globotriaosylceramide (Gb3) [3]. Roscoe Brady then showed that the lysosomal enzyme, α-galactosidase A (α-Gal A), was deficient in these patients [4]. Since that time, much has been learned about the clinical manifestations and molecular features of Fabry disease, now known to be the most common lysosomal storage disease. Two treatments, intravenous

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enzyme replacement therapy and oral molecular chaperone therapy, are clinically available to patients. Mouse and more recently rat models are now available to test new treatments and elucidate mechanisms of disease pathogenesis. This mini review provides an update to our understanding of Fabry disease mechanisms and treatments.

Clinical picture

The clinical picture of Fabry disease is characterized by progressive signs and symptoms that impact multiple organ systems (Table 1). The “classic” presentation of patients with no or minimal residual α-Gal A activity will be described here. However, it is important to note that the presentation is often heterogeneous, with a subset of patients experiencing phenotypes in only one (e.g., cardiac variant) or few organ systems. As Fabry disease is X-linked, males present with the classical manifestations more frequently than females.

In childhood, patients develop acroparesthesia, which consists of neuropathic pain in their distal extremities. Diffuse pain attacks and crises can also occur, lasting from minutes to days and are often precipitated by rising body temperature due to exercise, fever, or warm ambient environments [5]. Compounding this problem, patients frequently have sweating abnormalities, with the most frequent being anhidrosis or hypohydrosis. Ophthalmologic opacities, such as cornea verticillata and cataracts, are detected in childhood, but many patients retain intact vision (reviewed in [6]).

During their teenage years, patients develop angiokeratomas in the “bathing trunk” region. Proteinuria, a sign of kidney disease, may be detected at this time. Additionally, gastrointestinal distress, such as frequent and painful bowel movements, begins to affect patients. In adulthood, patients are at significant risk of end-stage renal disease, heart dysfunction (e.g., hypertrophic cardiomyopathy, cardiac arrhythmias, valvular disease), and cerebrovascular events (e.g., transient ischemic attacks, ischemic strokes). Patients may also develop osteopenia or osteoporosis [16,17]. With nephropathy being a major complication of Fabry disease, frequent dialysis treatments become a necessity. Neuropathic pain subsides in some adult patients, but many adults continue to live with debilitating pain (reviewed in [18]). Some adult patients display a unique neuropsychiatric phenotype, characterized by subtle movement impairment and depression [19]. Together, these numerous signs and symptoms significantly reduce quality of life [20].

Fabry disease is considered an attenuated lysosomal storage disease because patients survive into adulthood. However, patients lacking α-Gal A activity exhibit an ~10–20 year shortened life span: male patients with Fabry disease have a median survival of 57 years, and the female median survival is 72 years [21]. Many patients with Fabry disease do not develop symptoms until their teenage or adulthood years. Moreover, as neuropathic pain is a prominent symptom, patients are frequently misdiagnosed with more common diseases, such as fibromyalgia or rheumatologic pain diseases [22]. Although cornea verticillata and angiokeratomas are useful clinical signs, unfortunately many patients experience what they describe as a “diagnostic odyssey” in that while symptoms are prevalent and debilitating, diagnosis is delayed for long periods of time.

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The prevalence of Fabry disease was once believed to be rare: approximately 1:50,000 (reviewed in [23]). However, recent newborn screening efforts reveal that the incidence is much more common. In Italy, an incidence of 1:3100 was found [24] and in Taiwan, an incidence as high as 1:1250 was documented [25]. Studies in the United States report incidences of 1:5495 and 1:8454 in Washington and Illinois, respectively [26,27]. The overall discrepancy in estimated prevalence and measured newborn screening incidence is likely caused by the heterogeneity of clinical presentation, suggesting that newborn screening would improve the diagnosis and treatment of patients with Fabry disease. Given these findings, the overall patient population will increase as more states and countries include Fabry disease in their newborn screening panels.

Enzymatic defect

Fabry disease is caused by deficiency of α-Gal A, a lysosomal enzyme encoded by the *GLA* gene on the X-chromosome (region Xq22.1). Currently, there are 967 different *GLA* mutations, including 671 missense/nonsense mutations, listed on the Human Gene Mutation Database [28]. Many of these mutations are “private” in that they are only seen in one family. Having only one X-chromosome, males that are hemizygous for a pathogenic *GLA* mutation usually develop signs and symptoms, and males with completely deficient α-Gal A activity are impacted most severely, resulting in the “classic” presentation of symptoms. However, unlike other X-linked disorders, heterozygous females may experience significant disease symptoms depending on their residual α-Gal A activity [22,29]. Females homozygous for *GLA* mutations are especially rare but have been reported [30].

Through hydrolysis, α-Gal A removes terminal α1,3- and α1,4-linked galactosyl residues from various glycoconjugates within lysosomes. The primary substrate is Gb3, which accumulates to a major extent when α-Gal A is deficient. Minor α-Gal A substrates include deacylated Gb3 called globotriaosylsphingosine (lyso-Gb3), digalactosylceramide (Gal_2Cer), and blood group B and P1 glycosphingolipids (Figure 1). While lyso-Gb3 accumulates to a lesser extent compared to Gb3, it serves as a biomarker for monitoring therapeutic efficacy and contributes to the pathology of disease [31,32].

The crystal structure of α-Gal A was solved in 2004 by Scott Garman’s group [33]. α-Gal A exists as a dimer, and each monomeric unit of α-Gal A is composed of two domains: an N-terminal $(\beta/\alpha)_8$ domain and a C-terminal β-domain [33]. The active site of α-Gal A is located in the N-terminal domain and requires two key aspartate residues, D170 and D231, for hydrolysis of terminal α-linked galactose residues from the glycosphingolipid substrates. Three N-linked glycosylation sites are also present on each α-Gal A monomer, and two of these glycans are predominantly modified with mannose 6-phosphate [34,35], which is essential for transport to lysosomes by mannose 6-phosphate receptors (reviewed in [36]).

There may be mechanisms independent of α-Gal A capable of clearing α-galactosyl glycoconjugates. One example is the lysosomal enzyme, α-*N*-acetylgalactosaminidase (α-NAGA). In addition to hydrolyzing α-*N*-acetylgalactosamine from glycoconjugates, α-NAGA also contains α-galactosidase activity [37]. In fact, α-Gal A and α-NAGA are the only enzymes with α-galactosidase activity known to exist in humans [38]. Thus,

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endogenous α-NAGA may be able to partially compensate for α-Gal A deficiency. Supporting this possibility is the observation that α-NAGA is able to hydrolyze Gb3 in vitro [37,39]. Further, GSL loading experiments demonstrate that Fabry patient fibroblasts have a ~50% capacity to digest blood group B and a ~15% capacity to digest Gb3 [40], which is probably accomplished by α-NAGA. Although α-NAGA may partially compensate for α-Gal A, this putative compensation is clearly overwhelmed with age in patients with Fabry disease.

Biological significance of Gb3

Gb3 is a glycosphingolipid that is known by several names, including ceramide trihexoside, CD77, P^k blood group antigen, and Burkitt lymphoma antigen. Synthesis of Gb3 occurs in the Golgi with the addition of α-galactose to lactosylceramide by Gb3 synthase [41] (Figure 1). This enzyme is also known as lactosylceramide 4-α-galactosyltransferase (encoded by *A4GALT*). Once synthesized, Gb3 is localized to the outer leaflet of the plasma membrane, where it is clustered in lipid rafts with its glycan portion facing the extracellular environment. Upon endocytosis and delivery to lysosomes, the glycan portion faces the lysosomal lumen, which contains the exoglycosidases, including α-Gal A, that are essential for turnover of this glycosphingolipid (reviewed in [42]). Humans deficient in Gb3 synthase are phenotypically healthy but have an increased risk of miscarriage (reviewed in [43]), suggesting that Gb3 may play a role in embryogenesis. Gb3 synthase mRNA is highly abundant in human heart and kidney [44,45], which explains, in part, why patients with Fabry disease experience dysfunction of these organs as they contain high levels of this glycosphingolipid. Despite its potential importance in Fabry disease treatment, no structures have been reported for Gb3 synthase.

Cell surface Gb3 is involved in infectious processes, such as Shiga toxin infection, which may result in hemolytic-uremic syndrome (reviewed in [46]). Gb3 is the cell surface receptor that the Shiga toxin family uses for cellular entry [47]. Shiga toxins include Shiga toxin itself, which is produced by *Shigella* dysenteriae, and Shiga-like toxins (i.e., verotoxins), which are produced by enterohemorrhagic *E. coli* serotypes O157:H7 and O104:H4. Shiga toxins bind Gb3 molecules on the plasma membrane where they are subsequently endocytosed and transported to the endoplasmic reticulum. Following transport to the endoplasmic reticulum, these toxins localize to the cytosol and inhibit the 28S subunit of rRNA (reviewed in [48]). Ultimately, ribosomal protein synthesis is inhibited, leading to apoptosis. Humans infected with Shiga or Shiga-like toxins may experience hemolytic-uremic syndrome. This disorder is initially characterized by bloody diarrhea, followed by hemolytic anemia, thrombocytopenia, and acute renal failure. Because they do not express Gb3, mice deficient in Gb3 synthase are insensitive to Shiga toxin infection [49]. Paradoxically, Fabry mice that overexpress Gb3 are protected from Shiga toxin compared to wild type mice [50]. This may be because excess Gb3 acts as a “toxin sink,” where toxin-insensitive cells that normally do not express high levels of Gb3 absorb Shiga toxin, directing this toxin away from toxin-sensitive cells (e.g., renal cells) [50].

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Mechanisms of pathogenesis

While much is known about the molecular defects and clinical sequelae of Fabry disease, the mechanisms by which substrate accumulation leads to cellular dysfunction remain less defined [51]. Because Gb3 is turned over in lysosomes, the inability to digest Gb3 results in its lysosomal accumulation. However, the impact of α-Gal A substrate accumulation on the development of disease symptoms has increasingly been shown to involve cellular structures beyond strictly the lysosome (Figure 2). Downstream effects, such as fibrosis (reviewed in [52]), inflammation (reviewed in [53]), and the generation of reactive oxygen species [54–56], also seem to play key roles in pathogenesis. Pathogenic mechanisms of Fabry disease are discussed briefly below.

α-Gal A deficiency may lead to the dysfunction of multiple cellular components (Figure 2). Gb3 accumulation has been observed in the plasma membrane of α-Gal A-deficient cells [57,58]. An increase in plasma membrane Gb3 has the potential to alter the activities of various membrane proteins and channels, likely impacting cellular function in a tissue dependent manner. For example, Gb3 accumulation correlated with increased levels of transient receptor potential vanilloid 1 and altered neuronal I_h and Na_v 1.7 currents, suggesting that alteration of these ion channels contributes to the development of pain sensitivity [8]. Additionally, altered activity of cation channel transient receptor potential ankyrin 1 has been reported in Fabry sensory neurons [9]. As in many other lysosomal storage diseases, autophagy impairment is also observed in Fabry patient cells [59,60]. Because autophagy is highly dependent on the formation and fusion of membrane-rich autophagosomes with lysosomes, the alteration of autophagic membranes resulting from α-Gal A substrate accumulation may contribute to the impairment of autophagic flux [61]. In addition, mitochondrial dysfunction may be a pathogenic feature as the activities of respiratory chain enzymes are decreased in fibroblasts from Fabry patients [62]. This raises the possibility that Gb3 accumulation affects mitochondrial function, either directly through accumulation within the mitochondrial membrane or indirectly by preventing mitophagy. Finally, induction of the unfolded protein response has been implicated in Fabry disease [63], which suggests dysfunction of the endoplasmic reticulum.

Substrate accumulation and organelle damage ultimately results in oxidative stress, inflammation, and apoptosis. The induction of oxidative stress results in part by the decoupling eNOS, which increases the generation of reactive oxygen species [56,64]. Gb3 accumulation also correlates with an increase in the release of the inflammatory cytokines IL-1β, IL-6, and TNF-α in patient plasma [65,66], with TNF-α accumulation being especially pronounced in patients experiencing pain crises [67]. Together, the increased oxidant burden and proinflammatory state associated with substrate accumulation likely contributes to symptom development through multiple mechanisms. Proinflammatory cytokines have been implicated as a predisposing factor for thrombosis [68], stimulating the release of soluble prothrombotic markers, such as von Willebrand factor, and upregulating the expression of endothelial adhesion molecules [69,70]. In fact, both increases in von Willebrand factor and the expression of endothelial adhesion molecules have been observed in patients and mouse models of the disease [71,72]. Gb3 accumulation also promotes cell death pathways [73]. In cell types with limited regenerative abilities (e.g., neurons,

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podocytes), the Gb3-mediated stimulation of apoptotic pathways may contribute to the characteristic symptoms (e.g., pain, podocyte disease) experienced by patients. As mesenchymal stem cells in particular have increased rates of apoptosis and senescence [73], Gb3 accumulation in the bone marrow could also alter normal hematopoiesis.

The Gb3 metabolite, lyso-Gb3, has been shown to play important roles in disease pathology. Lyso-Gb3 is formed by the deacylation of Gb3 by acid ceramidase [74] (Figure 1). The accumulation of lyso-Gb3 exacerbates disease pathology as it both inhibits α-Gal A activity and promotes the proliferation of smooth muscle cells [31], a factor that likely contributes to the increased intima-media thickness observed in Fabry patients [75]. Lyso-Gb3 has been shown to promote inflammatory signaling in cultured podocytes [76,77] and may also directly sensitize nociceptors [78], contributing to the renal disease and neuropathic pain experienced by patients, respectively. Overall, the deleterious mechanisms of α-Gal A substrate storage outlined here are likely both substrate and cell-type specific, complicating our understanding of the molecular pathways by which Gb3 storage initiates and maintains the signs and symptoms observed in patients.

Rodent models

α-Gal A-deficient rodent models have been generated to study Fabry disease pathogenesis (Table 1). The first published report of a Fabry mouse (*Gla* KO) model was in 1997 by the groups of Roscoe Brady and Ashok Kulkarni at the National Institutes of Health [79]. In their first study, the authors reported that while Fabry mice appeared clinically normal at 10 weeks of age, microscopic and biochemical evidence of glycosphingolipid storage was evident [79]. The authors subsequently examined aging Fabry mice; however, they concluded that no clinical signs of Fabry disease or organ failure were evident in aged (80-week-old) Fabry mice [13]. Another group independently generated a *Gla* KO mouse model and also found no organ failure in aged mice [7]. Subsequent Fabry mouse studies documented neuronal glycosphingolipid storage and altered somatosensory phenotypes in Fabry mice, which are indicative of a neuropathic pain. These studies, however, show conflicting results [80–83]. The unavailability of strain-matched, wild type control mice likely contributes to the discrepancies. Nevertheless, the presence of biochemical and microscopic phenotypes in Fabry mice was critical in the development of enzyme replacement [84] and chaperone therapies [85].

A hypothesis to explain the asymptomatic Fabry mouse is that glycosphingolipid profiles are different between mice and humans. In fact, levels of red blood cell globosides, including globotetraosylceramide (Gb4), Gb3, and lactosylceramide, are much lower in mice than humans [13]. In an attempt to elicit phenotypes, one group crossed a transgenic mouse overexpressing Gb3 synthase (Gb3Stg) [86] with the *Gla* KO mouse. The resulting *Gla* KO/Gb3Stg mice developed a significant neurological phenotype characterized by spontaneous tremors, slowed movements, gait disturbances, and a rounded back [87]. Further, *Gla* KO/Gb3Stg mice had a median survival of 27 weeks, with all mice spontaneously dying by 35 weeks of age. Given that patients do not experience the observed neurological phenotypes to the same degree and do not suffer from an extremely reduced life expectancy, it appears that

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the phenotypes in *Gla* KO/Gb3Stg mice may limit the translational relevance of this *Gla* KO/Gb3Stg mouse model in therapeutic studies.

Given the limitations of Fabry mouse models, our lab developed a Fabry rat model. This rat model was generated using CRISPR/Cas9 technology to delete the rat *Gla* gene. As such, littermate-matched wild type controls are available to compare with KO rats. Fabry rats are completely deficient in α-Gal A activity and demonstrate accumulation of α-galactosyl glycosphingolipids in all tissues analyzed. We showed that Fabry rats develop pain-like behavior and that alterations in the cation channel, transient receptor potential ankyrin 1, may contribute to the development of neuropathic pain [9]. In addition, we found a significant decrease in various complex and hybrid N-glycans, including sulfated and sialated hybrid N-glycans, in Fabry rat dorsal root ganglia [9]. This observation suggests α-Gal A deficiency affects N-glycan processing within the Golgi, possibly by substrate accumulation within the membranes of this organelle (Figure 2). We also demonstrated that Fabry rats recapitulate cardiorenal phenotypes documented in patients, such as renal tubule dysfunction and mitral valve thickening [14]. Fabry rats develop corneal and lenticular opacities, a common finding in patients [10]. Our preliminary studies also reveal that Fabry rats experience progressive hearing loss as they age. Studies are currently underway to further characterize phenotypes in Fabry rats and understand how glycosphingolipid accumulation results in these phenotypes. The symptomatic Fabry rat model is a useful adjunct to existing Fabry mouse models in continuing efforts to understand disease pathogenesis and to test new and existing therapies.

Therapies

Currently, two therapeutic modalities are available clinically for the treatment of Fabry disease: enzyme replacement therapy and chaperone therapy. Other strategies, such as substrate reduction therapy, mRNA-based therapy, and gene therapy are in development (Figure 1). Enzyme replacement therapy, which consists of systemic α-Gal A infusion, was the first approved treatment for Fabry disease. There are two available pharmaceutical preparations of recombinant human α-Gal A: 1) agalsidase alfa (Replagal by Shire) is produced by overexpression in human fibroblasts, and 2) agalsidase beta (Fabrazyme by Sanofi Genzyme) is produced by overexpression in CHO cells. Both preparations have similar glycosylation patterns, specific activities, and enzyme kinetics [34], and both have been shown to be clinically efficacious [88,89]. However, there is a 5-fold dose discrepancy as agalsidase alfa is approved at 0.2 mg/kg biweekly, and agalsidase beta is approved at 1 mg/kg biweekly [90]. Only agalsidase beta is currently available in the United States, initially approved by the FDA in 2003. Agalsidase alfa is available in several locations outside of the United States, such as the European Union, Canada, Australia, Mexico, and South American countries. Currently, pegunigalsidase alfa (PRX-102; a covalently crosslinked, PEGylated form of α-Gal A), is being evaluated for safety and efficacy in clinical trials (, active; , active; , recruiting). There remains an ongoing debate in the field concerning the optimal age of enzyme replacement therapy initiation, but it is suggested that earlier treatment results in better outcomes [91]. Additionally, a recent 5-year study of male patients aged 5–18years supports the efficacy of agalsidase beta at the FDA-approved 1 mg/kg biweekly treatment rather than at a reduced dosage [92].

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Despite the availability of enzyme replacement therapy, challenges remain for its use in Fabry disease. Enzyme replacement therapy is time consuming (i.e., hours required for infusion) and expensive (~$200,000 per patient annually), placing a significant burden on patients and the healthcare system. Further, enzyme replacement therapy may cause infusion reactions and is not effective in all patients, such as those with end-stage organ disease [93] or with antibodies to the recombinant enzyme [94]. Therefore, the development of improved treatment options is an important goal for many researchers in academia and industry.

Molecular chaperones are another promising therapeutic avenue for Fabry disease treatment. Some patients have single point mutations that result in misfolded α-Gal A. While the mutated enzyme may possess some residual activity, it may be prematurely destroyed by ER-associated protein degradation. Thus, promoting enzyme stability, such as by a small molecule chaperone, may serve as a treatment. The concept is that a ligand (i.e., molecular chaperone) of α-Gal A may occupy its active site, thereby promoting enzyme folding and stability. Once the α-Gal A-chaperone complex enters the lysosome, the chaperone is dissociated from the enzyme due to pH-sensitive conformational changes and α-Gal A is free to act on glycosphingolipid substrates [95]. In order for chaperone therapy to be effective, patients must have amenable mutations (i.e., non-null α-Gal A activity that can be improved by the chaperone). Currently, the chaperone, migalastat (Galafold by Amicus Therapeutics) shows clinical efficacy in patients with amenable *GLA* mutations [96,97] and this orally administered small molecule drug was recently approved for use in the United States.

Substrate reduction therapy is another option currently under investigation. Two small molecules are being or are about to be tested in clinical trials: ibiglustat (; completed) and lucerastat (; recruiting) (reviewed in [98]). Both ibiglustat and lucerastat inhibit glucosylceramide synthase, the enzyme that adds glucose to ceramide (Figure 1). Because glucosylceramide is a common precursor in the synthesis of many glycosphingolipids (e.g., globosides, gangliosides, lactosides, sulfatides), glucosylceramide synthase inhibition results in decreased Gb3 synthesis.

Glucosylceramide synthase inhibitors have been tested preclinically in Fabry cell and mouse models and in clinical trials for patients with Gaucher disease (glucosylceramidase deficiency). In patient-derived lymphoblasts, glucosylceramide synthase inhibitors depleted Gb3 by 70–80% and reduced Gb3 levels below those of controls in α-Gal A deficient mice [99,100]. Clinical studies with miglustat, an inhibitor of glucosylceramide synthase, reported significant adverse events, such as weight loss, diarrhea, poor appetite, and tremor [101,102]. These side effects are probably due to the fact that miglustat more potently inhibits other enzymes, such as lysosomal and non-lysosomal β-glucosylceramidase and intestinal disaccharidases (reviewed in [103]). Recent clinical trials using the more selective glucosylceramide synthase inhibitor, eliglustat, provide support for the safety and efficacy of substrate reduction using more specific inhibitors; however, some patients experienced mild-to-moderate abdominal pain, diarrhea, and abnormal nerve conduction studies [104]. As gastrointestinal distress and peripheral neuropathy are dominant symptoms in Fabry disease, the effects of glucosylceramide synthase inhibition need to be further evaluated in patients with Fabry disease.

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Glucosylceramide synthase inhibition also depletes glucosylceramide and lactosylceramide, glycosphingolipids which do not accumulate in Fabry disease. Moreover, gangliosides are highly abundant in the nervous system and are important for neuron function (reviewed in [105]). Because lactosylceramide serves as the critical precursor to gangliosides, lactosylceramide depletion might contribute to the neurological side effects (i.e., tremor, abnormal nerve conduction studies) observed with these drugs. Therefore, substrate reduction therapy that specifically targets Gb3 synthesis and avoids depletion of lactosylceramide may be a better option in these patients (Figure 1).

There are several other Fabry disease therapies on the horizon. Gene therapy will likely be a future therapeutic option for patients with Fabry disease (reviewed in [106]). In two trials (and), CD34+ stem cells are obtained from a patient and are engineered to express α-Gal A using a lentivirus vector. The transduced cells are then transplanted back into the same patient (i.e., autologous stem cell transplantation) with the goal that secreted α-Gal A will be taken up by the patient's other cells. Adeno-associated virus capsids are also being evaluated for gene therapy, and novel capsids are in development to improve α-Gal A expression in kidney, heart, and brain [107]. Moving forward, gene therapy for Fabry disease has the potential to systemically express α-Gal A in a manner that would be effective for the large number of genetic mutations that cause Fabry disease.

mRNA-based therapies are also likely to become available for Fabry disease as studies in Fabry mice and non-human primates were recently reported [108,109]. This is an attractive option because the translated α-Gal A would express native post-translational modifications, such as mannose 6-phosphate on N-glycans for lysosomal targeting. With new therapeutic options becoming available, we may discover that combination therapies provide maximal benefit. For example, recent studies show that chaperone therapy coupled with enzyme replacement therapy is more efficacious than either option alone [110,111].

Conclusion

The field has made remarkable progress in our understanding and treatment of Fabry disease. However, an incomplete understanding of disease pathogenesis still limits our ability to effectively treat patients with this debilitating disease. Enzyme replacement therapy and chaperone therapy are currently approved for Fabry disease; however, these treatments are not curative and at best slow progression of the disease. Improvements to these therapies and many other therapeutic options are in the pipeline. Existing treatments can be optimized in animal models, which are now available in both the mouse and rat. Further, new treatments can be conceived and developed as we continue to learn about disease pathogenesis using these models. These efforts offer hope to the patients who suffer.

Acknowledgements

This work was supported by National Institutes of Health grants R01DK042667, K12HL141954, and R21NS095627 (to NMD). JJM is supported by a National Research Service Award (F30DK113641) and is a member of the Medical Scientist Training Program, which is partially supported by T32GM080202. AJK is supported by a career development program in translational glycosciences (K12HL141954). The Fabry rat model was generated under NIH resource grant, R24HL114474. The content of this review is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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Highlights

- Fabry disease is now recognized as the most common lysosomal storage disease
- Patients experience neuropathic pain and kidney, heart, and cerebrovascular disease
- The mechanisms of disease pathogenesis are incompletely understood
- Mouse and rat models are available to understand pathogenesis and test therapies
- Enzyme and chaperone therapies are approved, and new treatments are in trials

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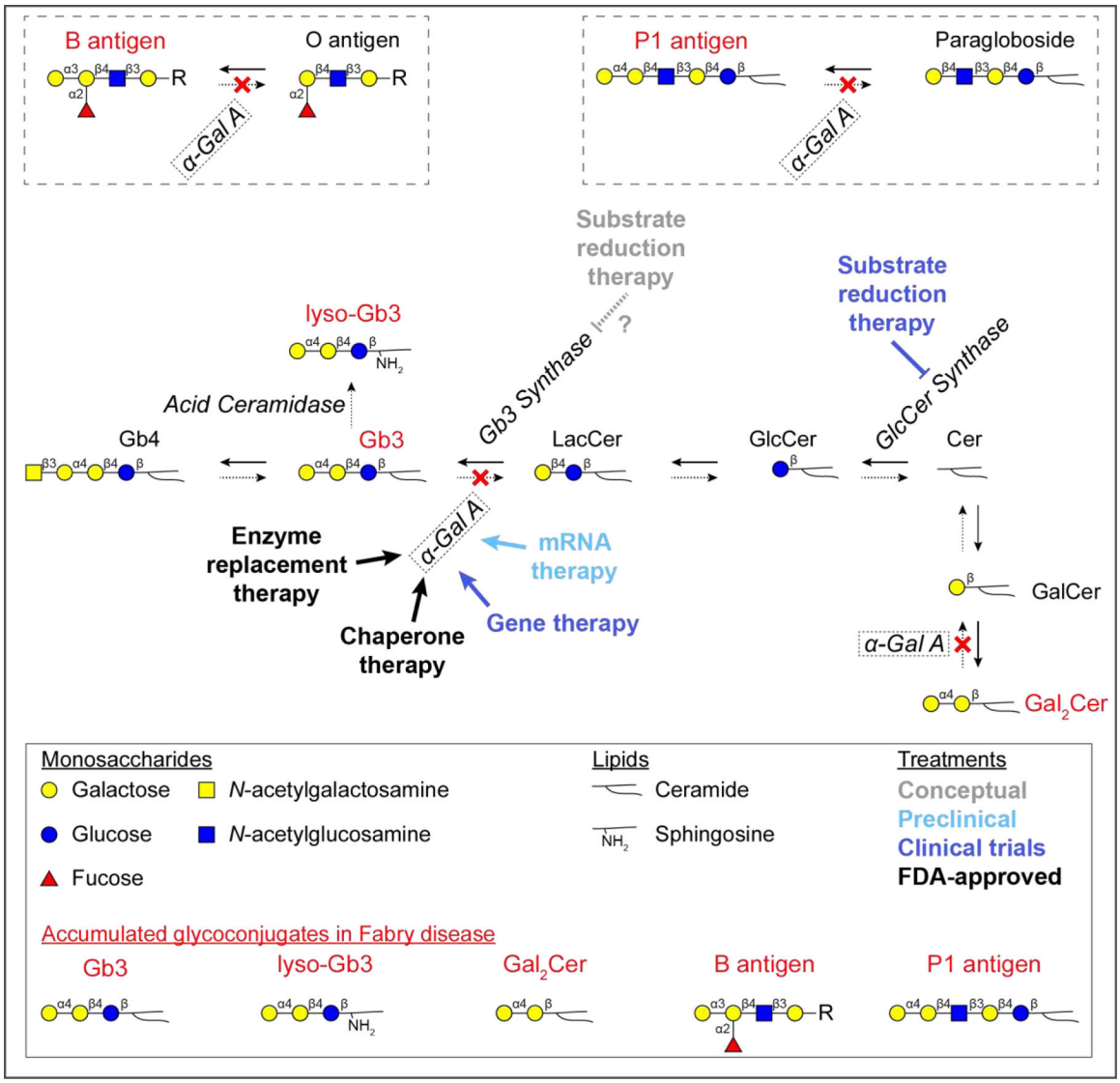

Figure 1: Accumulating glycosphingolipids and treatment targets in Fabry disease.

Glycosphingolipids are synthesized and degraded by sequential monosaccharide addition and removal, respectively. In Fabry disease, the lysosomal enzyme, α-Gal A, is deficient and glycosphingolipids with terminal α-galactosyl residues accumulate (red font). The major accumulating molecule is Gb3, but lyso-Gb3, Gal_2Cer, and blood group B and P1 antigens accumulate to a minor degree. Current and future therapies are aimed at replacing or promoting deficient α-Gal A activity. The FDA-approved therapies are enzyme replacement therapy and chaperone therapy. Clinical trials are currently underway to evaluate gene therapy and substrate reduction therapy. mRNA therapy is in preclinical development. Substrate reduction therapy trials are currently evaluating inhibitors of GlcCer synthase, which catalyzes a biosynthetic reaction prior to formation of Gb3, thereby reducing overall Gb3 load. However, this therapeutic strategy also inhibits the formation of GlcCer and LacCer, glycosphingolipids key to critical cellular processes, which ultimately results in an unfavorable side effect profile. Further, substrate reduction therapy inhibits the formation of

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other glycosphingolipids, such as gangliosides. Specific inhibition of Gb3 synthase may be more tolerable to patients with Fabry disease, but this approach is conceptual and remains to be developed and tested. The –R group represents that the B antigen glycan can be conjugated to either a protein or lipid. Abbreviations: globotetraosylceramide (Gb4), globotriaosylceramide (Gb3), globotriaosylsphingosine (lyso-Gb3), lactosylceramide (LacCer), glucosylceramide (GlcCer), ceramide (Cer), galactosylceramide (GalCer), digalactosylceramide (Gal_2Cer), α-galactosidase A (α-Gal A), glucosylceramide synthase (GlcCer synthase).

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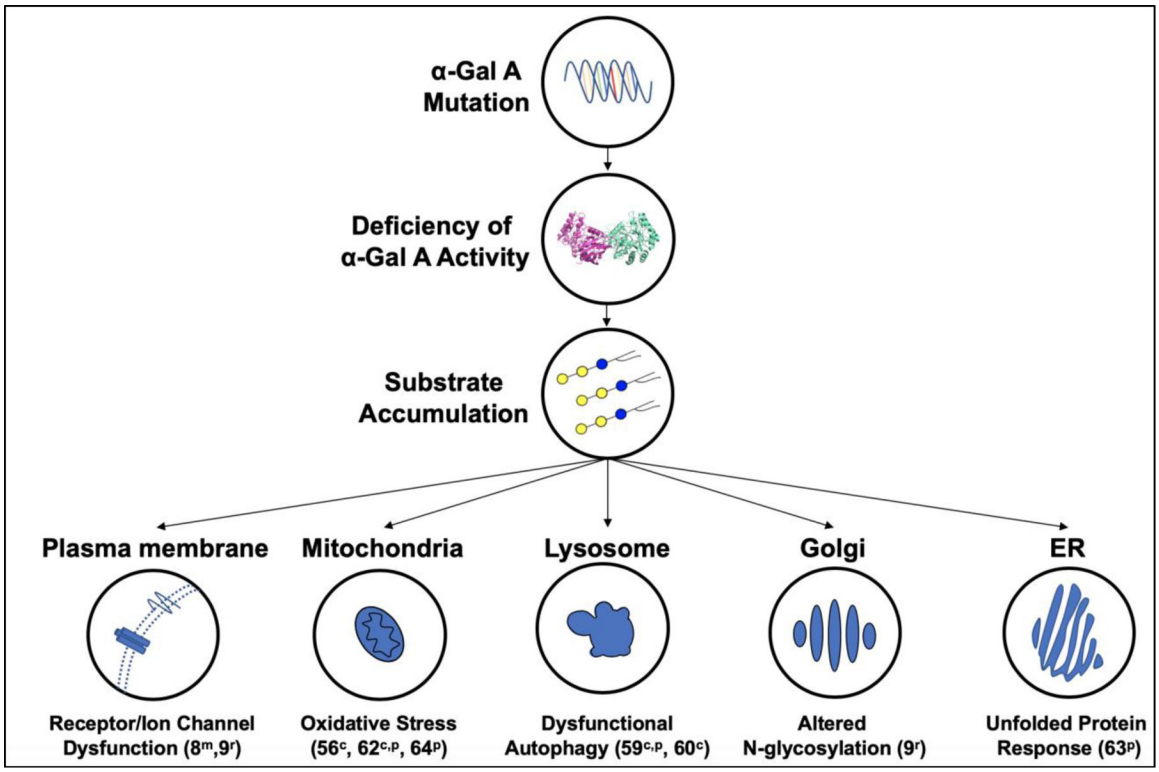

Figure 2: Cellular mechanisms of Fabry disease pathogenesis.

A pathogenic genetic mutation in the gene encoding α-Gal A causes decreased activity of this lysosomal enzyme. Subsequently α-Gal A substrates accumulate and lead to cellular dysfunction through multiple pathways. Substrate accumulation has been shown to alter the normal function of several subcellular components that are listed along with the corresponding effects observed in studies involving cells[c], Fabry patients[p], Fabry mice[m], and Fabry rats[r].

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Table 1:

Fabry disease signs and symptoms and animal model correlations

Sign or symptom[a]	Usual age of onset	*Gla* KO mouse	*Gla* KO rat
Acroparesthesia	Childhood	Yes [7,8]	Yes [9]
Chronic pain		Unknown	Unknown
Sweating abnormalities		No[b]	No[b]
Corneal & lenticular opacities		Unknown	Yes [10]
Hearing loss		No [11,12]	Yes (unpublished[c])
Gastrointestinal distress	Adolescence	Unknown	Unknown
Angiokeratomas		No	No[d]
Renal insufficiency	Adulthood	No [13]	Yes – tubular disease [14]
Cerebrovascular disease		Unknown	Unknown
Cardiac dysfunction/Cardiac arrhythmias		Debatable hypertrophy reported [15]	Yes – mitral valve disease [14]
Bone demineralization		Unknown	Yes (unpublished[e])

[a] Some patients, particularly those with substantial residual α-Gal A activity, may experience attenuated signs and symptoms or manifestations in only one organ system.

[b] Rodents do not sweat

[c] Beltrame, Runge, and Dahms

[d] While Fabry rats do not develop cutaneous vascular anomalies, evidence of macrophage infiltration and lipoatrophy is evident in Fabry rat skin [14]

[e] Miller and Dahms