

INVITED REVIEW ARTICLE

Gene therapy for glycogen storage diseases

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Abstract

The focus of this review is the development of gene therapy for glycogen storage diseases (GSDs). GSD results from the deficiency of specific enzymes involved in the storage and retrieval of glucose in the body. Broadly, GSDs can be divided into types that affect liver or muscle or both tissues. For example, glucose-6-phosphatase (G6Pase) deficiency in GSD type Ia (GSD Ia) affects primarily the liver and kidney, while acid α -glucosidase (GAA) deficiency in GSD II causes primarily muscle disease. The lack of specific therapy for the GSDs has driven efforts to develop new therapies for these conditions. Gene therapy needs to replace deficient enzymes in target tissues, which has guided the planning of gene therapy experiments. Gene therapy with adeno-associated virus (AAV) vectors has demonstrated appropriate tropism for target tissues, including the liver, heart and skeletal muscle in animal models for GSD. AAV vectors transduced liver and kidney in GSD Ia and striated muscle in GSD II mice to replace the deficient enzyme in each disease. Gene therapy has been advanced to early phase clinical trials for the replacement of G6Pase in GSD Ia and GAA in GSD II (Pompe disease). Other GSDs have been treated in proof-of-concept studies, including GSD III, IV and V. The future of gene therapy appears promising for the GSDs, promising to provide more efficacious therapy for these disorders in the foreseeable future.

Introduction

Glycogen storage disease (GSD), also referred to as glycogenosis, refers to a number of different diseases, all of which are caused by inherited abnormalities of enzymes that are involved in the formation or breakdown of glycogen (Table 1). These enzyme defects lead to abnormal tissue concentrations of glycogen or structurally abnormal forms of glycogen. The liver and muscle normally store glucose primarily as cytoplasmic glycogen, although lysosomal glycogen accumulates in Pompe disease. Individual GSDs may affect primarily the liver or muscle or both (Table 1). The liver GSDs usually present with hepatomegaly due to the inability to catabolize hepatic glycogen and frequently cause hypoglycaemia due to the lack of production of sufficient free glucose by the liver. Muscle GSDs frequently cause weakness and/or rhabdomyolysis due to skeletal muscle involvement and

sometimes result in cardiomyopathy related to heart involvement (Table 1). The severity of symptoms and lack of specific therapy have stimulated the development of new therapy for the GSDs, including gene therapy. Gene therapy has reversed disease involvement of both liver and muscle GSDs and continues to be developed as a new therapy for these disorders (Fig. 1).

Unmet clinical need for specific therapies in GSD

Need for therapies in GSDs I, III and IV. GSD type I (GSD I) is caused by defective glycogenolysis and gluconeogenesis and is classified into two subtypes: GSD Ia (Von Gierke disease/glucose-6-phosphatase/G6Pase deficiency) and GSD Ib (glucose-6-phosphate transporter/G6PT deficiency) (1–3). Lack of the G6Pase or G6PT enzyme results in impaired glucose homeostasis and hypoglycaemia (Table 1). Infants with GSD I present at age

Received: May 2, 2019. Revised: May 2, 2019. Accepted: June 7, 2019

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Table 1. Glycogen storage disorders (92–96)

Disorder	Eponym	Enzyme deficiency	Gene/inheritance ¹	Presentation
GSD Ia	von Gierke disease	G6Pase α	G6PC/AR	<ul style="list-style-type: none"> • Hypoglycaemia, hepatomegaly, short stature, hepatic adenomas, renal calcification and chronic kidney disease
GSD Ib		Glucose-6-phosphate translocase	SLC37A4/AR	<ul style="list-style-type: none"> • Symptoms of GSD Ia + neutropenia, inflammatory bowel disease and iron-resistant anemia
GSD II	Pompe disease	Acid α -glucosidase	GAA/AR	<ul style="list-style-type: none"> • Muscle weakness, hypotonia, cardiomyopathy and cardiorespiratory failure
GSD IIIa and IIIb	Cori disease	Debranching enzyme	AGL/AR	<ul style="list-style-type: none"> • IIIa: skeletal muscle weakness, cardiomyopathy, short stature, hypoglycaemia, hyperketosis, hepatomegaly and cirrhosis • IIIb: hypoglycaemia, hepatomegaly, hyperketosis, short stature and cirrhosis
GSD IV	Andersen disease	Branching enzyme	GBE/AR	<ul style="list-style-type: none"> • Skeletal muscle weakness, liver disease, short stature, hepatosplenomegaly, cirrhosis and cardiomyopathy
GSD V	McArdle disease	Muscle glycogen phosphorylase	PYGM/AR	<ul style="list-style-type: none"> • Exercise intolerance, cramps, myoglobinuria, second wind phenomenon and gout
GSD VII	Tarui disease	Phosphofructokinase	PFKM/AR	<ul style="list-style-type: none"> • Exercise intolerance, cramps and myoglobinuria
GSD IXa–IXd		Phosphorylase kinase	IXa: PHKA2/XL; IXb: PHKB/AR; IXc: PHKG2/AR; IXd: PHKA1/XL	<ul style="list-style-type: none"> • Liver (IXa–c): hepatomegaly, hypoglycaemia, short stature and hyperketosis • Muscle (IXd): exercise intolerance, cramps, myoglobinuria, skeletal muscle weakness and atrophy

¹AR means autosomal recessive; XL, X-linked.

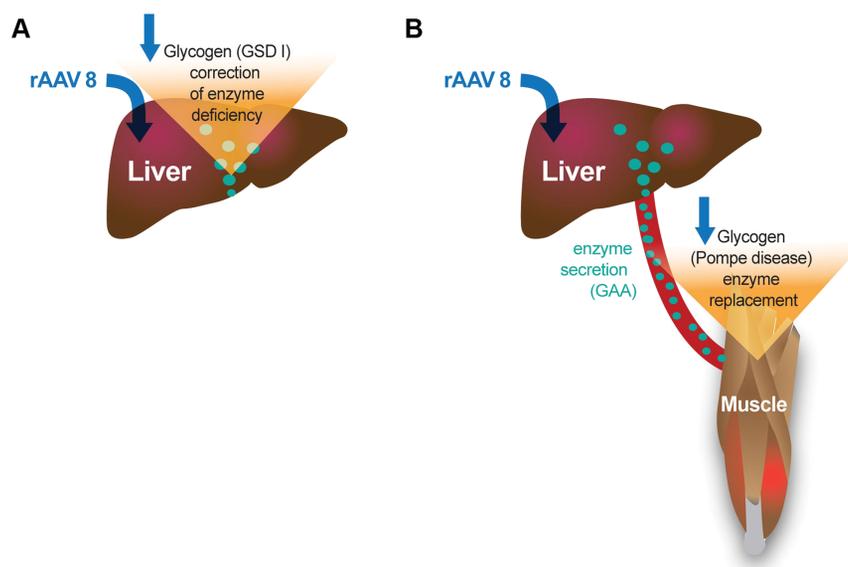


Figure 1. Liver-targeted gene therapy for GSD Ia and Pompe disease. Liver transduction with rAAV8 vectors has achieved biochemical correction through replacement of the deficient enzyme and glycogen clearance in the liver in GSD Ia (A) and from secretion of the therapeutic enzyme from liver accompanied by receptor-mediated uptake in the muscle in Pompe disease (B).

3–4 months with hepatomegaly, lactic acidosis, hyperuricemia, hyperlipidemia, hypertriglyceridemia, and/or hypoglycemic seizures. Glycogen and fat accumulation in the liver, kidney and intestines can result in long-term complications despite dietary management (Table 2).

GSD III, also known as Cori Disease or Forbes disease, is caused by deficiency in the glycogen debrancher enzyme (GDE) resulting in impaired glycogen breakdown (4–7). Abnormally structured glycogen accumulates in the liver, skeletal and cardiac muscles. Similar to GSD I, initial presentation occurs at a young age and commonly involves hepatomegaly, fasting hypoglycemia and hyperlipidemia (Table 1). While lactic acidosis is

not present, creatine kinase (CK) and liver transaminases may be significantly elevated, and the kidney is not involved. Despite dietary modifications, progressive liver disease often results in progressive liver fibrosis leading to cirrhosis, with the risk of developing hepatocellular carcinoma and/or liver failure. Long-term complications may also involve dilative cardiomyopathy or life-threatening arrhythmias and death (Table 2).

GSD IV (Anderson disease or brancher deficiency) is caused by reduced glycogen branching enzyme (GBE) activity, resulting in accumulation of abnormal glycogen which is plant-like (amylopectin) and is classified broadly as hepatic and neuromuscular forms (5,8,9). The progressive hepatic subtype is most common

Table 2. Current treatment and persistent symptom sequelae in GSDs I, II, III and IV

	Current treatment strategies	Examples of persistent symptom sequelae
GSD I (1,3,10,11,97,98)	<p>Symptomatic management and surveillance</p> <ul style="list-style-type: none"> • Dietary management to maintain normal glucose levels and prevent hypoglycemia and lactic acidosis, e.g. cornstarch therapy • Allopurinol (xanthine oxidase inhibitor) to prevent gout when blood uric acid concentration is not completely normalized via dietary therapy • ACE inhibitors or angiotensin-receptor blockers for hyperfiltration and/or albuminuria • Citrate supplementation for hypocitraturia • Thiazide diuretic for hypercalciuria • G-CSF administration in neutropenic patients (GSD Ib) and drugs to treat Crohn's-like ileitis • Lipid-lowering medications when lipid levels remain elevated despite good metabolic control • Treatment of liver adenomas with percutaneous ethanol injections, radiofrequency ablation and partial liver resection; liver transplantation when other interventions have failed • Kidney transplant in the setting of renal failure • Combined liver and kidney transplant in certain situations 	<ul style="list-style-type: none"> • Hepatic adenomas associated with significant risk of transforming into HCC • Long-term complications of kidney glycogen and fat accumulation: proteinuria, hyperuricemia associated with renal stones, renal tubular acidosis and kidney failure • Increased risk of osteoporosis, anemia and polycystic ovarian syndrome • Pulmonary hypertension
GSD II (5,13,14,18–20,99)	<p>ERT, with multisystemic approach to care (as needed)</p> <ul style="list-style-type: none"> • Immune modulation to prevent or eliminate immune response to ERT • Respiratory muscle training for respiratory insufficiency; noninvasive ventilation (BiPAP or CPAP) or invasive ventilation if needed • Physical therapy to optimize motor function; use of assistive devices if needed • Pharmacological chaperones and/or additional supplementation (e.g. CoQ) 10 to optimize ERT efficacy • Beta 2 agonists to optimize ERT uptake via M6P receptors • High-protein diet and other dietary interventions as needed 	<ul style="list-style-type: none"> • Persistent immunological challenges with ERT associated with severe clinical decline • Persistent manifestations despite ERT including <ul style="list-style-type: none"> ○ Cardiac arrhythmias ○ Respiratory insufficiency ○ Motor deficits ○ CNS and peripheral nervous system features (white matter lesions in the brain, small fiber neuropathy, bulbar weakness, dysphagia etc) ○ Vascular complications such as brain aneurysms, persistent ptosis, strabismus and myopia
GSD III (4,6,7)	<p>Symptomatic management and surveillance</p> <ul style="list-style-type: none"> • High-protein diet with cornstarch therapy • Liver transplantation for patients with severe hepatic cirrhosis, liver dysfunction, and/or hepatocellular carcinoma • Cardiac transplantation in patients with cardiac failure to cardiac fibrosis 	<ul style="list-style-type: none"> • Hypertrophic cardiomyopathy • Progressive liver disease, resulting in liver fibrosis and in some cases cirrhosis, adenomas and HCC • Progressive myopathy, osteoporosis and osteopenia • Risk of polycystic ovary disease • Neuropathy
GSD IV (8)	<p>Symptomatic management and surveillance</p> <ul style="list-style-type: none"> • Liver transplantation for patients with progressive hepatic subtype • Physical therapy for children with skeletal myopathy and/or hypotonia • Potential consideration of cardiac transplant for those with severe cardiomyopathy • Dietary management to prevent nutritional deficiencies 	<ul style="list-style-type: none"> • Hepatic subtype (most common): progressive hypotonia, progressive liver dysfunction and cirrhosis with hypoalbuminemia, leading to death by liver failure by age 5 years without liver transplantation • Congenital neuromuscular subtype: profound hypotonia, respiratory distress, dilated cardiomyopathy and death in early infancy typically due to cardiopulmonary compromise • Childhood neuromuscular subtype: mild to severe myopathy and dilated cardiomyopathy, with progressive course in some patients resulting death in the third decade

and is characterized by presentation with hepatosplenomegaly and liver cirrhosis within the first 18 months of life (Table 1). Liver failure is the ultimate outcome leading to death typically by age 5 years without liver transplantation. Neuromuscular subtypes of the disease are characterized by hypotonia, myopa-

thy, cardiomyopathy and nervous system dysfunction. The onset of these symptoms can occur from early infancy to childhood or later on in life [adult polyglucosan body disease (APBD)]. Significant morbidity and early mortality occur despite dietary therapy.

Currently, treatment strategies in GSDs I, III and IV are symptomatic and rely on multisystem surveillance with no targeted pharmaceutical intervention to treat the primary manifestations of the disease. While liver disease varies in severity across these three disease types, liver transplantation is ultimately the only intervention to correct severe hepatic damage. In GSD I, renal replacement therapy with dialysis or transplantation is considered in the case of advanced chronic kidney disease failing to respond to metabolic control or other interventions. In GSD III, patients have been reported with liver, kidney and heart transplant. In GSD I and GSD III, meticulous dietary therapy with small, frequent feedings and avoidance of fasting is the core principle to disease management (3,6,10,11). Raw, uncooked cornstarch is used to prevent hypoglycemia, especially overnight. Complex carbohydrates (as opposed to simple sugars) are preferred. A high-protein diet is recommended in GSD III but not in GSD I where kidney damage is a concern. Other interventions can be taken in GSD I to treat persistent symptoms such as lactic acidosis, hyperuricemia, neutropenia, hyperlipidemia and microalbuminuria (Table 2) (11).

There is a well-demonstrated need for therapies across GSDs I, III and IV. While dietary therapy and vigilant disease monitoring for specific symptoms can help prevent acute metabolic emergency in GSDs I and III, the progressive symptom sequelae of these diseases warrants therapies that can avoid the onset of severe complications such as hepatocellular carcinomas (HCC) (as well as hepatocellular adenomas (HCA) and renal failure in GSD I; liver cirrhosis in GSD III). In the case of patients with GSD IV, disease progression is severe to the extent that it may very likely lead to death during infancy or childhood. In those with the hepatic subtype, liver transplantation is the only option for rescue, as patients develop progressive liver cirrhosis without it (2,8).

Unmet needs despite treatment with enzyme replacement therapy in Pompe disease (GSD II)

GSD II, also known as Pompe disease, is caused by impairment in the lysosomal enzyme acid α -glucosidase (GAA) (5). It is classified as two general subtypes, infantile and late onset, with a spectrum of disease involvement in between. Infantile Pompe disease (IPD) is rapidly progressive; patients with IPD typically present in the first few months of life with severe hypotonia, respiratory distress and hypertrophic cardiomyopathy, among other complications (Table 1). Late-onset Pompe disease (LOPD) is typically slowly progressive primarily involving skeletal muscle without severe cardiomyopathy and encompasses those presenting later than infancy as well as the adult-onset form of the disease.

Enzyme replacement therapy (ERT) with recombinant human GAA (rhGAA, Myozyme and Lumizyme) is the only currently approved treatment for Pompe disease (12). The advent of ERT has significantly prolonged survival and improved clinical outcomes in patients with Pompe disease, especially in the infantile form, which was previously known to be fatal by the second year of life when untreated. Despite treatment with ERT, however, there remains a multitude of persistent complications that significantly limit clinical outcomes.

One significant challenge with ERT is the risk of developing antidrug antibodies (ADAs) to ERT, which have a severe deleterious effect on treatment efficacy and can lead to rapid clinical decline. Patients with cross-reactive immunological material negative IPD have the highest risk of developing ADAs, as they are completely unable to form endogenous GAA enzyme,

resulting in an immunogenic response to the therapeutic protein (13,14). Additionally, as patients with IPD are surviving well into adolescence, and patients with LOPD are being identified earlier through diagnostic advancements such as newborn screening (15), a new natural history is revealing persistent manifestations of the disease despite treatment with ERT (16,17). For example, one limitation is the insufficient or lack of glycogen clearance in certain tissue types, such as smooth muscle across vascular, ocular, gastrointestinal and respiratory systems (18). Reports have also revealed white matter lesions in the brain, which was previously thought to remain unaffected in Pompe disease (19,20).

Although some of these challenges can be resolved to a certain extent through close disease monitoring, adjunctive therapies to ERT and a multi-systemic approach to care, there is a demonstrated need for new therapies that can successfully target these specific manifestations.

GSD Ia

Transient efficacy from adeno-associated virus vector-mediated gene therapy in GSD Ia. Adeno-associated virus (AAV) vectors will express G6Pase in the liver, improving the abnormalities of GSD Ia. AAV vector administration to young mice accomplished a high level of liver transduction (Fig. 1), followed by declining numbers of vector genomes over the ensuing months (21–24). This phenomenon reflected the episomal nature of AAV vector genomes that are lost as cells divide during growth and development. For example, an AAV2 vector cross-packaged as recombinant AAV8 (rAAV8) vector decreased from >2 copies per liver cell at 1 month of age to 0.3 copies at 7 months of age in $G6pc^{-/-}$ mouse model of GSD Ia (21). Similarly, an rAAV8 vector was administered to a GSD Ia puppy at one day of age and prevented hypoglycemia for 3 h at 1 month of age; however, by 2 months of age the dog became hypoglycemic after 1 h of fasting and retreatment with a new rAAV1 vector was needed to restore efficacy (23). A larger study demonstrated greatly prolonged survival in GSD Ia dogs following treatment with repeated AAV vector administration using a new serotype for each treatment; however, those vectors failed to prevent progression of liver or kidney involvement from GSD Ia (25,26).

Gene editing with zinc finger nucleases to stably express G6Pase in GSD Ia. Integrating AAV vectors have been developed for the treatment of GSD Ia (27). One AAV vector contained zinc finger nucleases (ZFNs) that target sites within the ROSA26 locus, which has no adverse effect when interrupted and therefore represents a 'safe harbor' for transgene integration (28). The AAV-ZFN vector safely generated DNA breaks in the ROSA26 gene, which allowed integration of the AAV-G6Pase vector by homologous recombination to integrate the G6PC-derived transgene. Without the ZFN, integration occurs at random breaks in chromosomal DNA at a lower rate (29). The goal of this experiment was to integrate a human G6Pase expression cassette in the murine ROSA26 safe harbor locus using ZFNs to achieve sustained levels of G6Pase in the $G6pc^{-/-}$ mouse model (27). This approach to gene editing markedly improved survival and biochemical correction of $G6pc^{-/-}$ mice, in comparison with $G6pc^{-/-}$ mice treated with a non-integrating AAV vector (27).

Synergistic effect from gene editing and bezafibrate-induced autophagy in the GSD Ia liver. Treatment with the peroxisome proliferator-activated receptor (PPAR)-agonist bezafibrate in GSD Ia mice lowered glycogen and triglycerides in liver (30).

Therefore, we tested whether bezafibrate would enhance the efficiency of ZFN-mediated gene editing (27) by normalizing autophagy in the GSD Ia liver (31). The bezafibrate treatment group showed increased survival rate after AAV administration and decreased liver size (liver/body mass, $P < 0.05$), in comparison with other control groups (31). Bezafibrate with gene editing decreased liver glycogen and increased G6Pase activity and prevented hypoglycemia during fasting. Furthermore, bezafibrate-treated mice had a higher number of vector genomes, and ZFN activity was higher. Bezafibrate treatment normalized the impaired molecular signaling in GSD Ia as follows: (1) the expression of PPAR α , a master regulator of fatty acid β -oxidation; and (2) the expression of PPAR γ , a lipid regulator signaling. Therefore, bezafibrate improved the hepatic environment and increased the transduction efficiency of AAV vectors in liver, while higher expression of G6Pase corrected molecular signaling in GSD Ia. Thus, the benefits from stimulating autophagy during gene editing were two-fold: (1) from reversing the hepatosteatosis of GSD Ia (31), and (2) from increasing AAV vector transduction (32).

Correction of biochemical abnormalities and decreased tumorigenesis risk following AAV vector-mediated gene therapy. The prevention of HCA and HCC were described by Lee *et al.* in a long-term study of G6pc $^{-/-}$ mice treated with an rAAV8 vector containing a large G6PC promoter sequence to drive G6PC expression (AAV-GPE) (33). However, at the time no G6pc $^{-/-}$ mice had been documented to form HCA due to very high mortality in absence of gene therapy (34). The study did report that gene therapy mediated by the vector maintained efficacy for up to 90 weeks for mice expressing >3% of wild-type hepatic G6Pase- α activity, and that hepatic G6Pase activity decreased by >90% between 6 and 18 months. The treated mice displayed normal hepatic triglyceride content, had normal blood glucose in response to a glucose tolerance test, had decreased fasting blood insulin levels and maintained normoglycemia over a 24 h fast. A comparison between AAV-PE with another rAAV8 vector containing a minimal G6PC promoter sequence (AAV-G6Pase) (34) revealed higher transgene expression from the large G6PC promoter sequence in AAV-GPE (35). The high-level G6Pase activity achieved with AAV-GPE might explain the remarkably high efficacy achieved from only few cells expressing G6PC in the liver. The same group reported a threshold for prevent of HCA and HCC, when 3 G6pc $^{-/-}$ mice expressing 0.9–1.3% of normal hepatic G6Pase activity developed HCA/HCC (36). Kim *et al.* reported the activation of hepatic AMPK/sirtuin-1 and downregulation of STAT3/NF κ B-mediated inflammatory and tumorigenic signaling pathways to explain the prevention of hepatic tumors by >1% of normal G6Pase activity (37). Therefore, it was not surprising that HCC tumor formation was observed in GSD Ia dogs treated with AAV vectors encoding G6Pase because the AAV vector transduction in the GSD Ia canine model has been consistently <1% in multi-year follow-up (25,38). A somewhat conflicting report that AAV vector-mediated gene therapy completely prevented HCA/HCC formation for >5 years in dogs with GSD Ia, which can be understood in light of the 24 h nutrition provided in that study, which apparently markedly decreased the risk for tumorigenesis (26,38). Tellingly, the latter study reported a lack of tumor formation in GSD Ia dogs treated only with nutrition (no gene therapy) for >5 years, confirming that intensive nutritional therapy can prevent the progression of hepatic abnormalities in GSD Ia. Given the success of gene therapy with AAV-GPE, a Phase I clinical trial is currently underway with that vector (NCT03517085).

Gene therapy for GSD Ib partially effective due to lack of integration of AAV vector genomes. GSD Ib is complicated by neutropenia associated with increased risk for infection and related to the deficiency of glucose-6-phosphate transporter (G6PT), in addition to the liver and kidney involvement characteristic of GSD I (39). This myeloid dysfunction has resisted AAV vector transduction, which is readily understood related to the episomal status of AAV vector genomes that leads to loss of vector genomes during cell division (40). Consistent with this prediction, AAV vector-mediated gene therapy has reversed hepatic involvement and hypoglycemia when transduction was sufficient. An AAV vector containing the G6PC promoter/enhancer to drive G6PT expression revealed that G6pt $^{-/-}$ mice expressing 3–62% of normal hepatic G6PT activity featured the correction of hepatic involvement and glucose metabolism. However, 2 of 12 mice expressing <6% of normal hepatic G6PT activity developed HCA. Neutropenia persisted despite the reversal of biochemical abnormalities in these mice with GSD Ib, which suggested that episomal AAV vector genomes containing G6PT were lost from rapidly dividing neutrophils. Consistent with this hypothesis, G6pt $^{-/-}$ mice treated with a non-integrating adenoviral vector containing G6PT demonstrated the transient reversal of neutropenia, hypoglycaemia and hepatic glycogen storage that persisted for a few weeks (41).

Pompe disease

Liver depot gene therapy to systemically correct biochemical abnormalities. Amalfitano *et al.* demonstrated that high-level liver expression from a modified adenovirus vector produced circulating GAA in the blood, accompanied by receptor-mediated uptake in the heart and skeletal muscle (42). Although the GAA expression for liver proved to be transient, adenovirus vector-mediated GAA expression from the liver depot achieved high-level biochemical correction throughout the heart and skeletal muscle (43). Adenovirus vector-mediated gene therapy provoked anti-GAA antibodies that interfered with the biochemical correction of muscle (43). However, anti-GAA antibodies could be reduced by including a liver-specific regulatory cassette to drive GAA expression (44). Overall, these studies confirmed high-level production of GAA in the blood corrected the heart and skeletal muscle through cation-independent mannose 6-phosphate receptor (CI-MPR) mediated uptake of precursor GAA and trafficking to the lysosomes, where GAA was processed and cleared stored glycogen.

More recently AAV vectors have developed to produce secreted proteins including coagulation factors and lysosomal enzymes (45–47), including GAA in Pompe disease (48). The potential for liver depot gene therapy with AAV vectors to surpass ERT was demonstrated by studies that corrected GAA deficiency (Fig. 1) and extensively cleared lysosomal glycogen in skeletal muscle (42,43,48). Importantly, liver depot gene therapy can correct type II myofiber muscles that resist correction from ERT (49,50). Later studies suggested the feasibility of clearing sequestered glycogen from the central nervous system (CNS) following high-level hepatic GAA production (51,52), which can be attributed to CI-MPR-mediated transfer of a lysosomal enzyme such as GAA across the blood–brain barrier (53). The minimum effective dose for an AAV vector to achieve biochemical correction has been reported as 8×10^{10} vector genomes (vg) per kg body weight for the heart and diaphragm and 8×10^{11} vg/kg for skeletal muscle (54), although these minimum effective dosages will depend upon vector design. One advantage of gene therapy over ERT stems from the continuous,

low-level exposure of skeletal muscle to GAA from the liver depot, in contrast to periodic, high-level exposure from ERT (54).

Immune tolerance induction to GAA through liver-specific expression. The concept of AAV vector-mediated liver-specific transgene expression to suppress antibody responses against therapeutic proteins was developed first in animal models for hemophilia (55,56) and later in Fabry disease (47) and Pompe disease (48,57). Immune tolerance to GAA was induced by liver-specific expression, which was confirmed by the absence of anti-GAA antibody formation following vector administration (57–59). Furthermore, low-dose AAV vector administration could induce immune tolerance to GAA that enhanced the efficacy from simultaneous ERT (57,60). The induction of immune tolerance to GAA improved the biochemical correction from simultaneous ERT and prevented hypersensitivity reactions by suppressing anti-GAA antibody formation. The underlying mechanism is the activation of regulatory T cells that suppress antibody responses against GAA latter, which has been termed immunomodulatory gene therapy (61).

Promoter choice affects efficacy from gene therapy. The high tropism of AAV vectors for the liver reduces the dose requirements for gene therapy in Pompe disease. Muscle-targeted gene therapy has been attempted by incorporating a highly active muscle-specific regulatory cassette (MHCK7) in an AAV vector encoding GAA (62), but dose requirements were high. The dose needed to substantially clear lysosomal glycogen from skeletal muscle was high for a recombinant (r) AAV9 vector containing MHCK7 ($>2 \times 10^{13}$ vg/kg) in adult *Gaa*^{-/-} mice with Pompe disease (62), much higher than the highly effective dose for an rAAV8 vector containing a liver-specific promoter in the same strain of mice (2×10^{12} vg/kg) (54). A similar dose requirement was reported for an rAAV9 vector containing a desmin promoter at a higher dose (4×10^{13} vg/kg), confirming the high-dose requirements for direct muscle transduction in Pompe disease (63). AAV vectors containing constitutive promoters, such as an rAAV8 vector containing a cytomegalovirus enhancer/chicken β -actin (CB) promoter, provoked cytotoxic T lymphocyte responses and anti-GAA antibodies that prevented biochemical correction (48). Intriguingly, these immune responses against systemic GAA expression with a constitutive promoter can be suppressed by simultaneous administration of an AAV vector containing a liver-specific promoter to induce immune tolerance to GAA (59,64).

Methods to enhance liver depot gene therapy in Pompe disease. Gene therapy can be enhanced by methods to increase GAA secretion from the liver or to increase CI-MPR expression in skeletal muscle. The initial study of enhancing secretion of GAA modified the signal peptide of GAA to one from a highly secreted protein to produce a chimeric, secreted GAA (65,66). High-level hGAA was sustained in the plasma of mice with Pompe disease for 24 weeks following administration of an rAAV8 vector encoding chimeric GAA; furthermore, GAA activity was increased and glycogen content was significantly reduced in striated muscle and in the brain (66). These data confirmed the feasibility of modifying GAA to drive secretion from transduced hepatocytes, thereby increasing the availability of GAA for the cross-correction of skeletal muscle.

A more recent study of chimeric GAA confirmed the strategy of modifying the signal peptide (52). In this study the GAA cDNA was codon-optimized to increase GAA expression; however, anti-

GAA antibodies were detected in response to liver expression of the codon-optimized GAA even at moderately high-vector dosages (5×10^{11} vg/kg and 2×10^{12} vg/kg). Another vector containing GAA that combined both modifications of altering the signal peptide and codon-optimization successfully avoided anti-GAA formation at those dosages. These initial studies suggested that efficacy can be increased from a chimeric, codon-optimized GAA, if immune tolerance to GAA can be achieved.

Another strategy to enhance gene therapy in Pompe disease consists of inducing expression of CI-MPR in skeletal muscle (50,67). Treatment with the long-acting, selective β_2 -agonist clenbuterol increased CI-MPR in skeletal muscle and in the brain. The efficacy of liver depot gene therapy was enhanced by the addition of clenbuterol, as demonstrated by increased rotarod latency, in comparison with vector alone. Glycogen content was lower in skeletal muscles following combination therapy, including the tibialis anterior containing mainly type II myofibers, in comparison with vector treatment alone (50). Consistent with this preclinical data, a Phase I clinical trial revealed improved muscle function and biochemical correction following clenbuterol treatment in addition to ERT, in comparison with ERT alone, for adult patients with Pompe disease (68).

Potential reversal of nervous system involvement from Pompe disease with gene therapy. Lim *et al.* recently reported that an rAAVPHP.B vector can robustly transduce both the nervous system and skeletal muscles in infant *Gaa*^{-/-} mice (69). The rAAVPHP.B vector containing human GAA under the control of the CB promoter produced widespread GAA at supraphysiologic concentrations in the brain and heart, and glycogen content was significantly decreased in the brain, heart and skeletal muscle following vector administration. This biochemical correction correlated with the normalization of neuromuscular function. Although the rAAVPHP.B vector would not transduce human tissues and would not be effective in a clinical trial, this proof-of-concept data demonstrated the unprecedented reversal of muscle and nerve involvement with an AAV vector (69).

Phase I clinical trial of liver depot gene therapy for Pompe disease. A Phase I clinical trial of liver depot gene therapy for Pompe disease has begun enrolling adult patients (NCT03533673). This study will evaluate an rAAV8 vector containing a liver-specific promoter to drive wild-type GAA expression. Rather than frequent infusions of a recombinant protein, as in ERT, gene therapy with an rAAV8 vector will be performed once with long-lasting effects. Given proof-of-concept studies, it is anticipated that this strategy will induce specific immune tolerance to GAA in Pompe disease with a low dosage of this rAAV8 vector that expresses GAA only in liver, rAAV8-LSPhGAA (57,60). Preclinical experiments revealed a minimum effective dose of 2×10^{11} vg/kg that partially corrected GAA deficiency in skeletal muscle and induced immune tolerance to GAA (54). This preclinical data justified a starting dose of 1.6×10^{12} vg/kg for the Phase I clinical trial, given that the established threshold for safety was 10-fold higher (51). The first cohort of patients has been enrolled in this ongoing study.

Gene therapy for GSDs other than type I and II

Recombinant AAV vectors are currently preferred vehicles for the delivery of gene expression cassettes for their favorable safety properties and robust transduction capabilities. Despite

the rapid advances in gene therapy for GSD I and GSD II in the past decades, limited studies have been reported for other GSDs.

GSD III. Mutations in the *AGL* gene cause genetic deficiency of GDE, resulting in excessive accumulation of glycogen with short outer branches (limit dextrin) in multiple tissues, predominantly in liver and muscle. Most patients (85%) have both liver and muscle involvements (GSD IIIa) while others have disease limited to the liver (GSD IIIb). Progressive hepatic cirrhosis and liver failure can occur with age (Table 1); hepatic adenomas and hepatocellular carcinoma have been reported in some cases (6,70–72). Muscle weakness is present during childhood, and progressive myopathy and cardiomyopathy are major causes of morbidity in adults (6,73–75).

A major hurdle toward developing AAV-mediated gene therapy for GSD III is the inability to package a gene expression cassette containing the large-sized (4.6 kb) human GDE (hGDE) cDNA into a single AAV vector, due to its small carrying capacity. To overcome this limitation, Vidal *et al.* (76) employed a dual overlapping AAV vector system to express hGDE in a mouse model of GSD III, and the results were plausible. This system consists of two AAV vectors: one contains the 5' portion of the hGDE cDNA fused with a promoter (GDE-HEAD) and the other contains the 3' portion of the hGDE cDNA with a polyadenylation signal (GDE-TAIL). The two vectors share an overlap sequence for homologous recombination of the two segments to form the full-length hGDE coding sequence *in vivo*. Co-administration of the dual AAV vectors containing a liver-specific human alpha-1 antitrypsin (hAAT) promoter each at a dose of 2×10^{12} vg/mouse into adult GSD IIIa mice restored the expression of hGDE and reduced glycogen level in liver, but muscles (cardiac and skeletal) were left unrectified (76). Substitution of the hAAT vector with the universal CMV promoter resulted in hGDE expression and glycogen reduction in the heart and skeletal muscles, but not in the liver, likely due to the inactivation of the CMV promoter in the liver. The use of a liver-active CMV enhancer chicken beta-actin promoter in the dual vectors improved liver correction and rescued muscle function (76). While promising, a major limitation of this approach is that a cell has to acquire both vectors by a high-dose vector administration and then rely on the low-efficiency reconstitution of the full-length hGDE cDNA through homologous recombination, which cannot achieve an efficiency comparable to a single vector system. In the same article, the authors also reported that administration of an AAV vector expressing a secreted form of human GAA (Pompe disease) significantly decreased glycogen accumulation in the liver, but this treatment failed to rescue glycemia and muscle function in GSD IIIa mice (76).

Recently Pursell *et al.* (77) demonstrated that inhibition of glycogen synthase 2 using a lipid-nanoparticle-mediated RNAi gene silencing approach effectively prevented glycogen synthesis, glycogen accumulation, hepatomegaly, fibrosis and nodule development in liver in a mouse model of GSD IIIa (77).

GSD IV. GSD IV is caused by the deficiency of GBE and characterized by the accumulation of a poorly soluble, amylopectin-like glycogen (polyglucosan bodies) in liver, muscle and the CNS (78–80). Most early onset patients with GSD IV die in infancy or early childhood due to severe hypotonia, respiratory distress, cardiomyopathy and/or progressive liver cirrhosis; adult onset GSD IV constitutes the majority of adult APBD (81–83). Liver transplantation is the only treatment option for GSD IV.

Recently Yi *et al.* reported a gene therapy study in a mouse model of adult form of GSD IV (84). An AAV9 vector that contains a universal CMV enhanced chicken beta-actin hybrid (CB)

promoter, the human GBE cDNA, and the human growth hormone polyadenylation sequence (AAV-GBE) were intravenously injected into 14-day-old GSD IV mice at a dose of 5×10^{13} vg/kg. At 3 months of age, GBE enzyme activity was highly elevated in heart, and significantly increased in skeletal muscles and the brain, but not in liver of the AAV-treated mice. Glycogen contents were reduced to wild-type levels in skeletal muscles and significantly decreased in the liver and brain (84). At 9 months of age, glycogen contents were significantly reduced in the quadriceps (–86.5%), gastrocnemius (–92.3%), liver (–63.6%) and brain (–22.8%) by the AAV treatment, despite that no elevated GBE activities were detected in these tissues. Consistent with measured GBE activities, the AAV vector copy number in the 9-month-old AAV-treated mice was $<1/10$ of that observed in the 3-month-old mice in most tissues, indicating a gradual loss of viral vectors over time (>90 loss of AAV vectors from age 3 to 9 months). Plasma biochemistry tests revealed an overall trend of decreased plasma enzyme activities of ALT, AST and CK at 9 months of age, suggesting an alleviation of damage in the liver and muscle from the AAV-GBE treatment (84). However, the same AAV treatment failed to achieve efficacy when mice were treated at an adult age (3 months; unpublished data), which was likely a result of cytotoxic T cell immune responses provoked by the transgene expression (human GBE). Currently, we are evaluating strategies to suppress or evade cellular immune responses during gene therapy in adult mice.

GSD V. GSD V, also known as McArdle disease, is caused by mutations in the *PYGM* that encodes for the muscle form glycogen phosphorylase (myophosphorylase) enzyme (Table 1). Patients are frequently detected in the second to third decade of life by exercise intolerance with muscle cramping accompanied by elevated serum creatine kinase (85–88). There is no effective treatment for this disease, but many patients are able to perform moderate, sustained exercise on a carbohydrate-rich diet with carbohydrate ingestion shortly before exercise (89,90).

To date, the only gene therapy study for the disease was conducted in an ovine GSD V model (91). Intramuscular injection of a modified adenovirus 5 or an AAV2 vector containing myophosphorylase expression cassettes under the control of a *Rous Sarcoma virus* or CMV promoter effectively transduced and expressed functional myophosphorylase in the muscle of GSD V sheep, but the activity of myophosphorylase waned over time in all the treated muscles (91).

Conclusions

Liver-targeted gene therapy with rAAV8 vectors has efficaciously corrected the glycogen storage of GSD Ia and Pompe disease in preclinical studies (Fig. 1). Proof-of-concept experiments have successfully reversed the effects of GSD in multiple animal models, although further optimization will be required to advance gene therapy to clinical trials for GSDs other than GSD Ia and Pompe disease. However, successful clinical trials in one or more GSDs will fuel optimism regarding the potential of gene therapy to treat many or all of the GSDs.

Funding

National Institute of Diabetes and Digestive and Kidney Diseases (R01DK105434-01A1 to D.D.K.); National Institutes of Health Grant from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (R01AR065873 to D.D.K.); Alice and Y. T. Chen Center for Genetics and Genomics (to D.D.K.).

Conflict of Interest statement

D.D.K. has served on a data and safety monitoring board for Baxter International, and he has received funding from Roivant Rare Diseases. D.D.K. received an honorarium and grant support in the past from Sanofi Genzyme and Amicus Therapeutics. D.D.K., P.S.K. and Duke University have equity in Asklepios Biopharmaceutical, Inc. (AskBio), which is developing gene therapy for Pompe disease. Additionally, P.S.K. has received research/grant support from Sanofi Genzyme, Valerion Therapeutics and Amicus Therapeutics; has received consulting fees and honoraria from Sanofi Genzyme, Amicus Therapeutics, Vertex Pharmaceuticals and AskBio; and is a member of the Pompe and Gaucher Disease Registry Advisory Board for Sanofi Genzyme, Amicus Therapeutics and Baebies. P.S.K., B.S. and D.D.K. have developed technology that is described herein. If the technology is commercially successful in the future, the developers and Duke University may benefit financially.

Acknowledgements

We would like to acknowledge inspiration and support from Dr Emory and Mrs Mary. Chapman and their son Christopher, and from Dr. John and Mrs. Michelle Kelly. We deeply appreciate the dedication shown by the staff of the Duke Department of Laboratory Animal Resources, as well as undergraduate students at the Duke University. We thank Dr Janice Chou at the National Institute of Child Health and Human Development for providing *G6pc^{-/-}* mice, and Dr Nina Raben at the National Institute of Arthritis, Muscle and Skin Diseases for providing *Gaa^{-/-}* mice.

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