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New horizons in understanding and treating Pompe disease – diagnosis and therapy. A literature review

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A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

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Abstract

Introduction and Objective. Pompe disease (PD) is a genetic and metabolic disorder caused by a mutation in the GAA gene, which leads to a deficiency of acid alpha-glucosidase and glycogen deposition in lysosomes. The aim of the study is to review the genetic mechanisms, diagnostic approaches, and potential treatments for PD to improve our understanding and develop effective interventions.

Review Methods. The review examines articles from databases like PubMed, Google Scholar, Clinicaltrials.gov, and NCBI. Meta-analyses, randomized controlled trials, and research articles on PD, diagnosis and therapy were included after initial analysis. More than 95% of the articles are less than eight-years-old.

Brief description of the state of knowledge. Pompe disease has been a known clinical condition for almost a century, presenting challenges for diagnosis and treatment. Diagnosis can be difficult due to its similarity to other neuromuscular disorders. However, confirmation of the diagnosis can be achieved through enzymology and molecular genetic testing. The current treatment for PD is enzyme replacement therapy using recombinant human alpha-glucosidase. Ongoing research aims to develop improved or new enzymes, as well as other treatments, such as gene therapy and substrate reduction strategies. Early diagnosis and treatment are crucial. Neonatal screening is recommended.

Summary. Despite advancements in understanding the pathogenesis of PD, the genetic mechanisms underlying its phenotypic variations remain unclear. Reporting cases to databases is crucial for unravelling the molecular basis of symptoms and improving patient outcomes. New therapeutic approaches, such as modified enzyme replacement therapies and gene editing, are essential for overcoming current limitations and improving treatment efficacy in PD.

Key words

Pompe disease, Glycogen Storage Disease Type II, genetic therapy, enzyme replacement therapy

INTRODUCTION

Pompe disease (PD), also known as glycogenosis type II or acidic maltase deficiency, is a rare genetic and metabolic disease caused by an autosomal recessive mutation in the GAA gene, which encodes acid alpha-glucosidase (GAA). This results in a deficiency or absence of GAA, leading to glycogen deposition in lysosomes throughout the human body [1,2]. PD was first identified in 1932 by pathologist Joannes C. Pompe, who described the symptoms of idiopathic cardiac hypertrophy in a seven-month-old female infant. In 1963, Henri-Gery Hers discovered GAA and established it as the cause of PD [1,3].

PD has two basic forms: infantile-onset (IOPD), which is characterised by GAA activity below 1%, and late-onset (LOPD), which has a less severe course of the disease [2,4]. The global prevalence of PD was previously estimated at 1 in 40,000 live births; however, newborn screening programmes (NBS) in different countries have revealed much higher figures, ranging from 1:4,447 – 1:37,094 [5,6].

In recent decades, there have been significant advances in the understanding of the pathophysiology of PD and the development of therapeutic strategies. The most significant advance in the treatment of the disease has been the introduction of enzyme replacement therapy (ERT) using recombinant GAA (rhGAA). While this therapy is not capable of completely halting progression of the disease, it markedly enhances the quality and length of life of patients. Nevertheless, modifications of ERT and attempts to utilize gene therapies that could improve treatment outcomes are still being developed. In order to achieve effective treatment of PD, it is important to be able to rapidly identify patients who are experiencing symptoms of the disease. This can be achieved through the use of newborn screening (NBS) management.

The aim of the study is to review the current understanding of the genetic mechanisms underlying PD, existing diagnostic approaches, and potential therapeutic interventions, including novel experimental treatments. A comprehensive analysis of these aspects is essential to improve our

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understanding of PD, and to advance the development of effective treatments.

MATERIALS AND METHOD

The review considers articles published in databases such as PubMed, Google Scholar, Clinicaltrials.gov, and NCBI databases. English language publications were included using search terms such as 'Pompe disease', 'etiology', 'diagnosis', 'enzyme replacement therapy' and 'gene therapy'. After a preliminary analysis, meta-analyses, randomized controlled trials, review and research articles of PD, diagnosis, and therapy of PD, were included. Over 95% of the articles are less than eight-years-old.

Clinical phenotypes – Infantile onset of Pompe disease (IOPD). IOPD is typically diagnosed shortly after birth or in the first few months of life [1]. If left untreated, the disease progresses rapidly, and patients usually become dependent on respiratory support at 4.7 months of age due to the involvement of the diaphragm and respiratory muscles. [3,7]. The initial symptoms of the condition include slowed movement with decreased muscle tone (52–96% of patients), hepatosplenomegaly (29–90% of patients), and macroglossia (29–62% of patients) [1,9]. IOPD is distinguished by cardiovascular involvement, manifesting as hypertrophic cardiomyopathy in approximately 90% of cases and left ventricular outflow tract closure. This results in a reduced ejection fraction and heart failure, which represents a significant cause of mortality in IOPD. [8–10]. Furthermore, cardiac arrhythmias, including supraventricular or ventricular tachycardia or premature beats, may be present [4,10].

The ability to attain certain motor milestones, such as sitting or standing, may be delayed or absent due to a congested muscular system. Gastrointestinal muscle weakness is linked to dysphagia, gastroparesis, and chronic constipation. Furthermore, hyporeflexia or areflexia, droopy eyelids, scoliosis, and winged scapula have been observed [8]. Abnormal lung ventilation can increase the risk of recurrent upper respiratory tract infections and pneumonia, and may contribute to sleep disturbances, particularly during REM sleep [7,8]

In the brain, there appears to be a reduction in myelin in the periventricular white matter, which can lead to seizures, encephalopathy and future cognitive impairment [11]. Hearing impairment, assessed by behavioural audiometry and evoked potentials, has been documented in some individuals [3,8].

Late-onset Pompe disease (LOPD). LOPD typically presents symptoms from infancy to adulthood, with the severity and age of onset dependent on the GAA variant and the degree of enzyme activity [1, 5]. The disease is characterized by the hallmark sign of progressive symmetric proximal myopathy, but the pattern of muscle involvement is variable and may be regional. The lower extremities and spinal muscles are usually affected earlier than other regions of the body. As the disease progresses, motor weakness leads to the development of scoliosis and lumbar hyperlordosis. In some cases, patients may develop stiff spine syndrome (RSS) [12,13].

Respiratory muscles are frequently affected in LOPD, and associated complications such as recurrent infections and respiratory failure represent a significant cause of mortality. [5,12,13]. Additionally, cardiovascular abnormalities, including cardiac arrhythmias and arterial aneurysms, including those in the arteries of the central nervous system (CNS), have been observed in patients with LOPD [12,13]. However, myocardial hypertrophy, a characteristic of IOPD, is rarely seen in patients with LOPD. [5,12]

Gastrointestinal symptoms, including dysphagia, diarrhea, faecal incontinence, abdominal discomfort, malabsorption and constipation, are prevalent and markedly impact on the quality of life of patients, and the prevalence of psychological disorders [12,13]. Psychological issues, including a reduction in social activity, anxiety and depression, are further exacerbated by the presence of chronic pain, fatigue, and incontinence [5,12]

Molecular genetic of PD. Pompe disease is an autosomal recessive disorder caused by mutations in the *GAA* gene which encodes the enzyme alpha-1,4-glycosidase (GAA). The primary function of the GAA enzyme is to catalyse the hydrolysis of the alpha-1,4-glucosidic chains of glycogen in the acidic pH environment of lysosomes. Consequently, a decrease in GAA activity leads to the accumulation of glycogen in the lysosome [1].

The *GAA* gene is located on the long arm of chromosome 17 (17q25.3) and is approximately 28 kb in length. It consists of 20 exons, including a non-coding first exon containing a 5'-untranslated sequence [1,2]. The GAA enzyme precursor is transported to the endoplasmic reticulum (ER) by amino-terminal peptide sequences. Here, it undergoes N-glycosylation before being transported to the Golgi complex. High-mannose oligosaccharide side chains are further modified to deliver GAA to lysosomes via the mannose-6-phosphate receptor (M6PR) [2]. The final processing produces a 70 kDa mature form of the enzyme.

The *GAA* gene is the only gene associated with PD. The 'Pompe disease *GAA* variant database' [14] provides a comprehensive list of reported *GAA* variants, accompanied by a severity classification system proposed by Kross et al. [14,15]. This system comprises six classes (A-F). The distribution of each class is shown in Figure 1, illustrating the frequency of their occurrence. In addition, the database contains information on cross-reactive immunological material (CRIM) status [14,16]. CRIM-negative status has been associated with poorer prognosis and response to ERT [16].

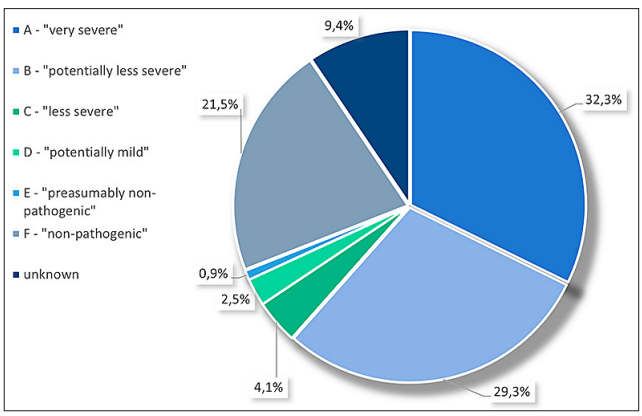

Figure 1. Frequency of a particular class of GAA variants reported in the Pompe disease GAA variant database [14]

The current database documents a total of 911 variants of mutations – 232 intronic and 678 exonic [14], two of which involve the deletion of the entire *GAA* gene. All types of mutations have been recorded, covering disorders related to protein structure formation. Missense mutations are the most prevalent, followed by small deletions [2]. Figure 2 shows the distribution of *GAA* variants based on their severity class and their localization in exonic and intronic regions.

The spectrum of mutations is heterogeneous and genetic variants often occur only in small populations, except for the splicing mutation c.-32–13T>G, which is the most prevalent in the Caucasian population [1,16]. This intronic mutation causes a splicing defect that results in the skipping of exon 2, reducing the level of synthesis of the correct enzyme. In homozygotes with this mutation, a variable level of residual *GAA* activity and a delay in the manifestation of phenotypes have been observed [16]. The most common mutations in the Chinese and Taiwanese cohort were c.1935C>A and c.2238G>C, while c.546G>T was over-represented in Japanese PD patients [17]. The c.2560C>T mutation has affected many African or African-American patients with IOPD [16].

The relationship between the level of GAA activity and phenotype in PD is complex and does not solely indicate the severity of the disease. Patients with GAA activity levels below 1% usually present with a severe form of IOPD. Conversely, the most common severity class in patients with LOPD is class D, with GAA activity levels between 5% – 30%. However, patients with IOPD with *GAA* variants indicating mild potentially class, have also been reported in the database [14,15]

Due to the lack of strict genotype/phenotype correlations and the variable response to treatment, researchers are searching for genes that modify GAA expression. Previous studies have suggested that the insertion/deletion (I/D) polymorphism of the ACE gene, which encodes the

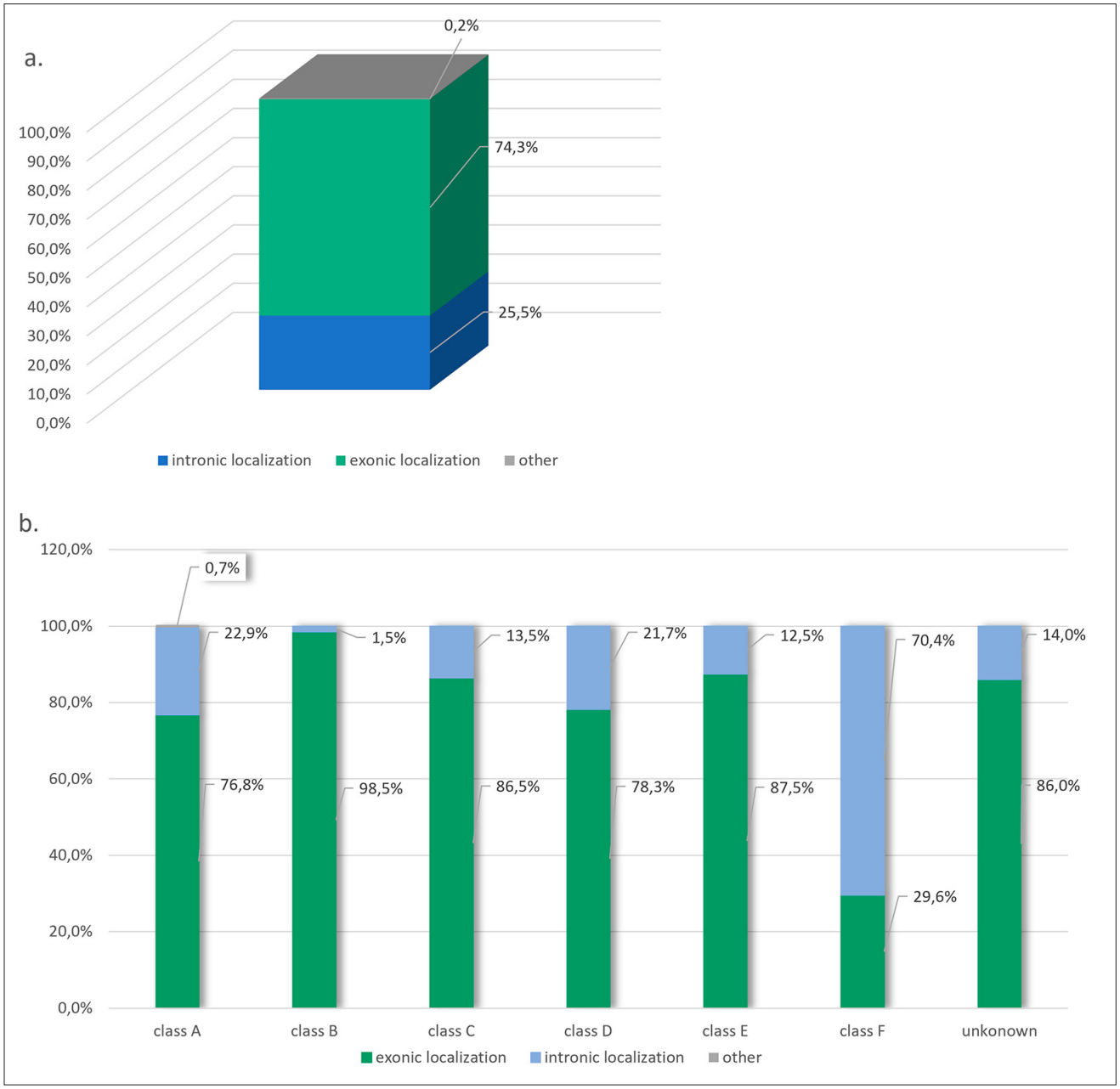

Figure 2. GAA genetic variants distribution into exonic and intronic localization in all reported in database (a) and in individual severity classes (b) [14]

angiotensin I-converting enzyme (ACE), may be a modifier of disease onset and/or response to enzyme replacement therapy (ERT). However, the association with ACE I/D polymorphism, as well as with other possible modifier genes, remains controversial [18].

Diagnostic tools. Rare diseases, such as PD, can present with symptoms that are difficult to recognize and can only be diagnosed through genetic testing. Unfortunately, the diagnostic process can take months, and in some cases this delay can lead to death before the cause is even known [1,3]. Similarly, LOPD patients may experience a slower onset of symptoms, which can prolong the diagnostic journey for years [3,5].

The primary method of diagnosing PD is to detect the absence of GAA activity in lysosomes and to identify mutations in the gene encoding the enzyme [1,19]. However, one of the challenges of enzymatic diagnosis is the presence of pseudo-deficiencies, such as *GAA* variants, which do not cause PD but reduce the activity of the enzyme [1]. GAA activity can be measured in leukocytes, dried blood spots (DBS) or fibroblasts from muscle or skin biopsies [20,21]. For efficient screening and diagnosis, it is highly advantageous to use DBS or leukocytes as they are easy to obtain. The guidelines for diagnosing PD recommend a combination of enzymatic assays confirmed by gene sequencing as the gold standard [1,19,20].

Muscle biopsy is a quite specific test for PD, and the diagnosis of PD may also be supported by a muscle biopsy. Histological examination may reveal certain features, such as the presence of vacuoles filled with PAS-positive material – glycogen and autophagic deposits containing large autofluorescent inclusions composed of lipofuscin, an indigestible intralysosomal material typically associated with the aging process [5,22]. Muscle biopsy can improve the understanding of response to treatment; for example, it has allowed the description of a previously unreported pathology that is ERT-resistant in cases of LOPD [5,22].

A useful diagnostic tool for the early identification of people with LOPD is spirometry, which is highly effective for detecting the signs of respiratory impairment commonly seen in LOPD. Forced vital capacity (FVC) should be measured in both the seated and supine positions. A decrease in FVC of more than 10% when changing position from sitting to lying down indicates diaphragmatic weakness, which may precede proximal myopathy and may qualify a patient for ERT before full-blown disease symptoms, and may also be used to assess response to treatment [19, 23].

Elevated levels of creatine kinase (CK), transaminases (alanine transaminase (ALT) and aspartate transaminase (AST) and lactate dehydrogenase (LDH) are sensitive but non-specific markers for LOPD [1]. CK levels are typically elevated in patients with LOPE, ranging from 1.5 – 15 times the upper limit of normal [23]. Persistent hyperCKemia accompanied by respiratory insufficiency and proximal muscle weakness should be further investigated for LOPD [20,24]. An increase in urinary glucose tetrasaccharide (Glc4) does not differentiate PD from other glycogen storage disorders; however, it may support the diagnosis when clinical findings are consistent and can be useful in monitoring treatment outcomes in clinical trials [1].

Another tool that can be used for monitoring the changes in PD is qMRI of the muscle, which is particularly valuable for monitoring disease progression and plays an important role in clinical trials and treatment follow-up. The technique is used to evaluate muscle fat infiltration and atrophy in PD is quantitative muscle MRI (qMRI). This non-invasive measure can visualize and quantify muscle degeneration [25]. Unlike conventional magnetic resonance imaging (MRI), qMRI can detect microstructural processes of tissue remodelling and damage, even in tissue that appears normal on conventional MRI imaging. [25,26].

Recent studies indicate that microRNAs may serve as a biochemical marker for PD. These non-coding RNA molecules play a crucial role in post-transcriptional gene expression regulation [27]. One specific microRN – miR-133a – is expressed in both skeletal and cardiac muscle. Analysis of plasma samples from PD patients showed significantly higher levels of miR-133a than in controls. According to the study, miR-113a levels were found to be higher in patients with IOPD than in those with LOPD [28]. For patients with IOPD, early diagnosis through ERT is crucial, and the diagnostic process needs to be accelerated. One solution to this problem, at least in part, is NBS. NBS was first introduced in Taiwan in 2005, and has since been implemented as a pilot or regular programme in several countries, including Japan, some regions of Italy, Australia, several states in the USA, Austria, Hungary, Mexico, and Brazil [1,21]. The NBS method measures GAA activity in DBS using techniques such as fluorometry, tandem mass spectrometry, or digital microfluidic fluorometry [20,21]. NBS is especially crucial in IOPD patients, as improved overall survival and mechanical ventilation-free survival in previously treated patients. Chien et al. analyzed the results of three cohorts which showed that patients detected with NBS had 100% overall and ventilator-free survival at 150 months. In comparison, later-diagnosed and ERT-treated patients had an overall survival of approximately 50%, and ventilator-free survival of approximately 20% at 150 months [20]. In countries with experience of NBS and improved diagnostic methods, the time from diagnosis to initiation of ERT has been significantly reduced, even up to one day after diagnosis [20,21].

In LOPD patients, the variability of phenotypes is high and the timing of symptom onset cannot be predicted at the time of diagnosis. Nevertheless, early detection by NBS allows close monitoring of patients with LOPD, increasing the likelihood of initiating ERT at the onset of symptoms [6,20]. Although NBS has been shown to provide many benefits, it has also been reported to cause psychological problems for families of LOPD patients. Many families reported feeling uninformed at the time of diagnosis and experiencing increased anxiety and hypervigilance to symptoms, highlighting the need for standardized psychological care [6,21].

An important limitation of NBS is that it cannot detect GAA deficiency, which means low enzyme activity in tests but no clinical symptoms of the disease. This relationship may account for the likely overestimation of PD incidence rates. Consequently, there is also a risk of over-treatment, which may not be beneficial to patients and may be costly in terms of time and resource [21]. It is crucial to highlight that NBS is not a diagnostic tool, therefore any positive result must be further verified as false positive results may occur.[19,21]

Treatment approaches – current therapeutic standards. ERT is the current standard treatment for PD [1,4]. This treatment

involves intravenous infusions of human recombinant alpha-glucosidase (rhGAA), which is transported to the lysosome via M6PR, enabling patients with LSD to metabolize glycogen properly [1,29]. The search for a cure for PD has a long history, dating back to the 1960s with the discovery of *GAA* [3,5]. Trials in patients were conducted using enzymes from human placenta and Aspergillus Niger, rabbit milk, and Chinese hamster ovary cells [19 – 31]. As a result, in 2006, alglucosidase alfa became the first rhGAA to be approved for both IOPD and LOPD [30,32].

Although ERT is generally considered safe, it can cause adverse effects which can be divided into two main groups: infusion-associated reactions (IARs) and the development of anti-rhGAA IgG antibody titers [30,33,34]. The most commonly reported IARs in patients receiving ERT are typically mild-to-moderate in severity and do not usually interfere with continued administration of ERT [19,30]. Premedication with antihistamines, corticosteroids and antipyretics or desensitization may be given in the event of IARs [1,19].

A more significant concern is the development of anti-drug antibodies (ADAs), which can either inhibit the activity of rhGAA or reduce its uptake by cells [34,35]. In particular, IOPD patients with negative CRIM status are more susceptible to deterioration in ERT efficacy due to the development of ADAs. CRIM status determines a patient's ability to endogenously synthesis GAA, with GAA produced by CRIM-positive patients being non-functional but inducing higher immune tolerance to rhGAA [35]. To prevent the development of ADAs and reduce their amount, a prophylactic immune tolerance induction (ITI) protocol can be administered, which includes methotrexate, rituximab, intravenous immunoglobulin, and sometimes bortezomib [19, 35]. It has been shown that ITI is more effective in ERT-naive patients and with a shorter duration of therapy. Therefore, it is crucial to determine a patient's CRIM status by Western blot analysis before starting ERT. Unfortunately, studies have shown that CRIM-positive PD patients can also develop ADAs [33].

Treatment guidelines suggest ERT at a dose of 20 mg/kg every two weeks [30,32,33] as soon as patients are diagnosed with IOPD [34,37], and at the onset of symptoms in LOPD with a confirmed diagnosis [19,32]. The standard dose of 20 mg/kg every two weeks was established based on the first pivotal clinical trials, as higher doses of ERT did not prove to be more effective. However, a multi-centre retrospective study of 28 IOPD patients revealed that those who initiated ERT earlier or received higher weekly or fortnightly doses, had a lower risk of motor decline [32,33]. A multi-centre observational study was conducted on a cohort of 116 IOPD patients. Higher doses were administered to classic IOPD patients and showed that those treated with 40 mg/kg weekly had a higher overall survival rate than those treated with the standard dose. However, the effect on motor function was not statistically significant [29]. Although these results suggest the possibility of dose escalation in routine ERT, more data is needed to support this, particularly in the adult cohort of LOPD patients [29,36].

ERT is currently the only internationally approved disease-modifying therapy for PD. However, PD is a multi-systemic disease and management should be multidisciplinary [1,4]. Medical care for PD patients should include appropriate immunization [4,10,12], consideration of non-invasive respiratory support and respiratory muscle training [1,4,34], with recent guidelines suggesting the inclusion of exercise under professional supervision [34]. Other treatments, such as an oral/nasogastric/gastrostomy tube, hearing aids and speech therapy should be considered [34].

Although the introduction of alglucusidase alfa offers hope for changing the prognosis for all people with PD, it is not a panacea. Heterogeneous responses have been observed in all forms of PD, and the health of most patients improves to some degree. However, studies show that IOPD patients lose motor milestones over time and about 50% of patients experience a decline in respiratory function [10,30]. Although cardiomyopathy is reversible in most patients within months to a year of treatment, other cardiac dysfunctions, such as arrhythmias, may occur. [10,31]. After an initial improvement in respiratory and motor function, most LOPD patients experience a regression or plateau [4,19].

Novel and experimental therapy methods. In the light of the unsatisfactory results of current treatments, particularly in improving respiratory and neurological function, there is a need for new therapeutic options [30,37]. Promising developments in this area include molecular modifications of the rhGAA and gene-modifying applications, such as adeno-associated viral (AAV) gene therapy and lentiviral (LV) vectors for *ex vivo* haematopoietic stem cell (HSPC) gene therapy. Table 1 presents an overview of clinical trials for gene therapy, while Table 2 displays current clinical trials for ERT.

ERT modifications. One possible strategy to improve the efficacy of ERT is to increase the binding of rhGAA to the cation-independent MP6R (CI-M6PR) [4,39]. This can be achieved by conjugating oligosaccharides involving bis-mannose-6-phosphate (bis-M6P), which is then attached to the rhGAA. This methodology has been employed to develop second-generation rhGAA therapeutics, including Avalglucosidase-alpha (Nexviadyme), which was approved by the Food and Drug Administration (FDA) in 2021 for the treatment of LOPD in patients aged one year and older [3,4] following positive results from a recent randomised, phase 3 trial (COMET) [39]. The recommended dose of avalglucosidase alpha is 20 mg/kg body weight administered once every two weeks [5,39]. Currently, there are two ongoing clinical trials (NCT03019406, NCT04910776) focusing on infants with IOPD. Another potential approach to achieve this goal is the incorporation of a glycosylation-independent lysosomal targeting (GILT) peptide tag consisting of a segment of insulin-like growth factor 2 (IGF2). The aim of this modification is to increase the affinity of rhGAA for the IGF2 binding site on the IGF2R/CI-M6PR receptor [37,39]. This method has been applied to BMN 701, a GILT-tagged rhGAA, and has been evaluated in open-label study (NCT01230801) and a phase 3 switchover study (NCT01435772), both involving intravenous infusion in LOPD patients. The results of these studies have shown improvements in respiratory function and walking endurance [38]

The bioavailability of rhGAA can also be improved by using small molecule chaperones to enhance the enzymatic activity of GAA. Chaperones facilitate the proper folding of the GAA protein, allowing it to maintain its catalytic activity and prevent premature degradation in the ER [40,41]. Cipaglucosidase alfa (Pombiliti™), an rhGAA product under development by Amicus Therapeutics in Philadelphia, USA, along with the

Table 1. Overview of clinical trials for gene therapy [38]

NCT number	Treatment	Status	Phase	Description
NCT02240407	rAAV9-DES-hGAA	completed	Phase 1	Randomized, controlled study evaluating the toxicology, biodistribution and potential activity of re-administration of rAAV9-DES-hGAA injected intramuscularly into the TA.
NCT03533673	AAV2/8-LSPhGAA (ACTUS-101)	active	Phase 1	Prospective, open-label trial designed to objectively assess the safety and bioactivity of ACTUS-101 in subjects diagnosed with LOPD
NCT00976352	rAAV1-CMV-GAA	completed	Phase 1/2	Trial of Diaphragm Delivery of Recombinant Adeno-Associated Virus Acid Alpha-Glucosidase (rAAV1-CMV-GAA) Gene Vector in Pediatric Subjects Aged 2 to < 18 Years with PD
NCT04093349	SPK-3006 (adeno-associated viral (AAV) vector)	active	Phase 1/2	Dose-escalation Study to Evaluate the Safety, Tolerability and Efficacy of a Single Intravenous Infusion of SPK-3006 in Adults With LOPD receiving ERT
NCT06178432	CRG003 (BBM-G102, AAV vector)	not yet recruiting	Early Phase 1	A Single-arm, Open-label, Single-dose Study to Evaluate the Safety, Tolerability, and Efficacy of CRG003 Injection in the Treatment of LOPD with a long-term follow-up period of 5 years.
NCT05567627	GC301 (AAV vector)	recruiting	Not Applicable	Single Arm, Multicenter, Open and Dose-escalation Clinical Study on Safety, Tolerance, and Efficacy of GC301, an AAV-Delivered Gene Transfer Therapy in Patients With IOPD
NCT05793307			Phase 1/2	A Multi-cantered, Single Arm, Open-Label, Study to Evaluate the Safety and Efficacy of an Adeno-associated Virus Vector Expressing the Human Acid Alpha-glucosidase (GAA) Transgene Intravenous Injection in Patients With IOPD
NCT04174105	zocaglusagene nuzaparvovec (AT845, AAV8-Delivered Gene Transfer Therapy)	recruiting	Phase 1/2	Open-Label, Ascending-Dose Clinical Study to Evaluate the Safety and Preliminary Efficacy of AT845, an AAV8-Delivered Gene Transfer Therapy in Patients With LOPD (FORTIS)

Table 2. Current clinical trials for ERT [38]

NCT number	Treatment	Status	Phase	Description
NCT04676373	Alglucosidase alfa (Myozyme)	recruiting	Phase 4	A Single Arm, Prospective, Open-label, Multi-center Study to Evaluate Efficacy and Safety in Chinese Patients With Late Onset Pompe Disease With Alglucosidase Alfa Treatment (APOLLO-LOPD)
NCT05164055	Avalglucosidase Alfa	Active, not recruiting	Phase 4	A French Multicenter Open Label Phase 4 Extension Study of Long-term Safety and Efficacy in Patients With Pompe Disease Who Previously Participated in Avalglucosidase Alfa Development Studies in France
NCT03019406		Active, not recruiting	Phase 2	An Open-label Ascending Dose Cohort Study to Assess the Safety, Pharmacokinetics, and Preliminary Efficacy of Avalglucosidase Alfa (NeoGAA) in Patients With IOPD Treated With Alglucosidase Alfa Who Demonstrate Clinical Decline or Sub-optimal Clinical Response (Mini-COMET)
NCT04910776		recruiting	Phase 3	An Open-label, Multinational, Multicenter Study of the Efficacy, Safety, Pharmacokinetics, and Pharmacodynamics of Avalglucosidase Alfa in Treatment naïve Pediatric Participants with IOPD (Baby-COMET)
NCT04532047	Aldurazyme (laronidase)	recruiting	Phase 1	In Utero Enzyme Replacement Therapy (ERT) for Prenatally Diagnosed Lysosomal Storage Disorders (LSDs) (IUTER) fetal ERT
NCT04138277	Cipaglucosidase Alfa/Miglustat [rhGAA (ATB200) co-administered with a chaperone (AT2221)]	active, not recruiting	Phase 3	A multicenter, open-label extension study of ATB200/AT2221 in adult subjects with LOPD who completed Study ATB200-03
NCT04808505		recruiting	Phase 3	An Open-label Study to Evaluate the Safety, Efficacy, Pharmacokinetics, Pharmacodynamics, and Immunogenicity of Cipaglucosidase Alfa/Miglustat in Both ERT-experienced and ERT-naïve Pediatric Subjects With Infantile-onset Pompe Disease Aged 0 to < 18 Years (ROSSELLA)
NCT03911505		recruiting	Phase 3	An Open-label Study of the Safety, Pharmacokinetics, Efficacy, Pharmacodynamics, and Immunogenicity of Cipaglucosidase Alfa/Miglustat in Pediatric Subjects Aged 0 to < 18 Years With LOPD
NCT03865836		available		An expanded access program (EAP) for eligible participants designed to provide access to ATB200/AT2221.
NCT02675465		active	Phase 1/2	An Open-Label, Fixed-Sequence, Ascending-Dose, First-in-Human Study to Assess the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and Efficacy in Adult Subjects With Pompe Disease

small-molecular chaperone miglustat, was granted approval in the European Union (EU) on 27 March 2023, as a two-component long-term ERT for the treatment of adults with LOPD [40]. The recommended dosage of cipaglucosidase alfa is 20 mg/kg administered intravenous every two weeks. The results of a phase I/II trial (NCT02675465) revealed that the combination of cipaglucosidase alfa and miglustat effectively reduced biomarkers of muscle damage (CK) and glycogen accumulation (Glc4) in patients with LOPD, particularly among those who had not received ERT. In the randomized, phase III PROPEL trial (NCT03729362), cipaglucosidase alfa plus miglustat did not achieve statistical superiority over alglucosidase alfa plus placebo in terms of improving the six-minute walk distance (6MWD) in patients with LOPD. However, the combination of cipaglucosidase alfa and miglustat demonstrated the potential for clinically meaningful improvements in motor and respiratory function, while maintaining a comparable safety profile to that of alglucosidase alfa plus placebo [40, 41].

An attempt has been made to deliver rhGAA to the cell via the equilibrative nucleoside transporter 2 (ENT2), independent of the CI-M6PR, using VAL-1221 (Valerion Therapeutics), a fusion protein of the lupus anti-DNA monoclonal antibody 3E10 with rhGAA (NCT02898753). However, a clinical trial was discontinued due to lack of funding [30]. Targeting the transferrin receptor (TfR) has also been explored as a way to improve efficacy across the blood-brain barrier (BBB). This is achieved by binding rhGAA to an anti-human TfR antibody, allowing transcytosis across the BBB and subsequent delivery to the CNS compartment [37].

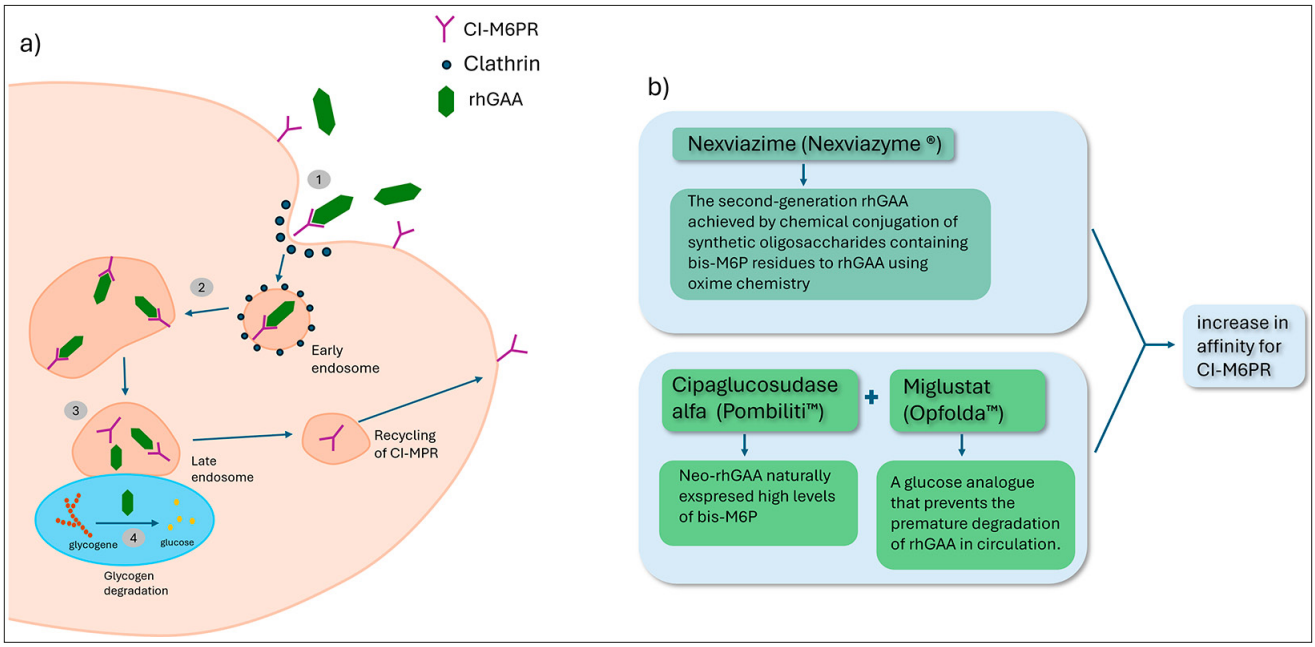

Figure 3. a) Overall mechanism of action of rhGAA therapy in the cell: 1. rhGAA binds to CI-M6PR on the cell surface, where it undergoes endocytosis via clathrin-coated pits. 2. The rhGAA and CI-M6PR complex is transported via the endocytic pathway to the early endosome. 3. Following the disconnection of CI-M6PR in a lowered pH environment of the late endosome, rhGAA is transported into the lysosome. 4. In an acidic lysosomal environment, glycogen is degraded by the active GAA form following proteolytic cleavage and N-glycan trimming. b) The general principle of the newly approved rhGAAs for the treatment of PD CI-MPR, cation-independent mannose-6-phosphate receptor; rhGAA, recombinant human acid alpha-glucosidase; bis-M6P, bis-mannose-6-phosphate [37,39,40,41]

Gene therapy for PD. The Adeno-associated virus (AAV) is a single-stranded DNA virus of the Parvoviridae family, characterized by a biphasic life cycle with productive and latent phases [42]. Successful gene therapy using recombinant AAV vectors has been demonstrated in murine models of PD [37].

In a preclinical study,**?** – delivered the AAV1-CMV-GAA vector within the diaphragm using a glycerine-based gel and demonstrated tropism of the AAV1 serotype in the expression of GAA across the diaphragm [37]. An important finding was the attenuation of efferent phrenic nerve activity, indicating retrograde transduction. This method has also been utilized in a clinical trial with IOPD patients, aimed at improving respiratory function, compared to baseline (NCT 00976352) [43]. Although satisfaction levels improved during the first 180 days, respiratory parameters began to deteriorate by day 365. To sustain the therapeutic effect, it may be necessary to administer a re-dose of the treatment.

To improve tissue-specific expression, especially in skeletal muscle, cardiac muscle, and motor neurons, a modified promoter for muscle creatine kinase (MCK) and desmin (DES) was utilized. In pre-clinical studies, a construct containing an AAV vector encoding human GAA expressed by MCK was administered into GAA-KO mice and non-human primates [37,44]. However, a high dose of this construct resulted in an immune response and subsequent heart failure. The rAAV9-DES-hGAA vector was used in randomized clinical trials (NCT02240407) after promising results from preclinical studies with DES promoter [37]. Patients with LOPD received intramuscular injections of the vector in the tibial anterior (TA) muscles [37,44].

A liver-specific promoter (LPS) derived from the thyroid hormone-binding globulin promoter and comprising an alpha-1 microglobulin/bikunin enhancer sequence was successfully utilised in preclinical studies with an AAV8-hGAA vector to reduce glycogen deposits in the heart and diaphragm [37]. These trials also confirmed the feasibility of using liver cells to produce recombinant proteins and reduce the immune response to transgenic products. In addition, studies using a hybrid tandem liver-muscle promoter (LiMP) and AAV8 and AAV9 serotypes injected into Gaa-/-mice showed a reduction in anti-GAA antibody production compared to ERT [37,44]. These findings led to the initiation of clinical trials to assess the safety of AAV2/8-LSPhGAA (ACTUS-101) in LOPD patients (NCT03533673). Various modifications have been introduced to increase GAA protein secretion by transduced liver cells and optimize transgene expression, such as the use of a specific human alpha1-antitrypsin (hAAT) signal peptide, or an N-terminal deletion fused to a codon-optimized GAA [37]. In a preclinical study involving non-human primates, the AAV8-hAAT-sp7-delta8-coGAA vector demonstrated increased hepatic GAA secretion, reduced immunogenicity, and increased GAA activity in plasma and skeletal muscle [37].

Neuropathology is a significant aspect of PD that requires consideration in CNS therapy. The rAAV9, which exhibits both muscle and CNS tropism, has been utilized in murine and non-human primate models [37,45]. Hordeaux et al. demonstrated an improvement in both neurological and cardiac function in Gaa-/-mice following intrathecal administration of AAV9-CAG-hGAA [45]. Similar results were observed in a study on mice models using the newly isolated AAV-B1 serotype [46]. Preclinical studies have shown a satisfactory therapeutic effect in the brain and spinal cord using the synapsin I (Syn-I) promoter construct for neuron-specific expression (yfAAV9/3-Syn-I-hGAA) [37,47].

Lentiviral-mediated HSPC gene therapy has also been employed to treat LSD. The method consists of *ex vivo*-transduced HSPCs engrafting into the bone marrow niche and differentiating into the full spectrum of haematopoietic cells and microglia in the CNS [37,46]. Pre-transplant bone marrow conditioning, including irradiation and

chemotherapeutic agents, is required [37]. Stok M. et al. reported positive outcomes in a preclinical study by using an LV expression cassette. This resulted in increased GAA activity, reduced glycogen accumulation in the heart and skeletal muscles, and detectable levels of GAA protein in microglia and astrocytes, suggesting cross-correction [48].

Alternative molecular-based strategies, such as chemical modification of antisense oligonucleotides (AONs) that are insensitive to RNase-mediated degeneration, have shown promise in preclinical settings by enhancing the endogenous production of wild-type *GAA*, correcting aberrant splicing, and mediating inclusion [49]. This approach, however, only benefits a small subset of patients with similar gene variants.

Additionally, there may be concerns about compromising safety, including the ability to mount an immune response to the capsid protein and transgene product, pre-existing neutralizing antibodies to AAV, inflammatory toxicity resulting from increased vector doses, complement activation, cytopenia, and hepatotoxicity [42,49]. To address these concerns, short DNA oligonucleotides can be incorporated into vector genomes to antagonize TLR9 activation. This may be beneficial in reducing TLR9-mediated immune responses, allowing for higher doses to be infused into patients [37].

Furthermore, the use of CRISPR tools has led to the development of novel mouse models that more accurately mimic human disease phenotypes. For instance, the IOPD model utilizes sgRNA CRISPR-Cas9 homology-directed recombination to harbour the orthologous GAA mutation c.1826dupA (p.Y609*) [37,50]. These models may offer valuable insights into the safety and efficacy of rAAV-mediated gene transfer. Recent preclinical studies have used PD rats generated by CRISPR/Cas to investigate the use of a novel highly myotropic bioengineered capsid AAVMYO3 and an optimized muscle-specific promoter in conjunction with a transcriptional cis-regulatory element. Treatment with AAVMYO3-Gaa vectors resulted in a widespread expression of GAA in muscle throughout the body. This resulted in a decrease in glycogen storage, an increase in muscle strength, prevention of cardiomegaly, and an improved survival rate [50].

SUMMARY

Although Pompe disease has been recognized as a clinical entity for almost a century, it remains a diagnostic and therapeutic challenge for clinicians. Significant progress has been made in understanding the pathogenesis over the years, but the genetic mechanisms responsible for the diversity of phenotypes are still not fully understood. Therefore, it is important to report cases to the database to better understand the molecular basis of the pathomechanism of symptoms and to analyze the prospects for patients. The NBS, which has been introduced in many countries, is an important element in reducing the time from diagnosis to treatment initiation. ERT is an undisputed success in PD, but it has limitations, including its inability to affect the CNS and the immune response, which requires frequent re-dosing. To overcome these limitations, further research and new therapeutic techniques are needed. Modified ERTs and gene therapy are important areas of research. However, it is crucial to thoroughly evaluate factors such as dose level, biodistribution, and immunogenicity. Technological advances in vector design and transgene editing are necessary to enhance target tissue coverage. Gene editing can be challenging due to the wide range of mutations found in patients. Future therapeutic regimens may be influenced by new techniques for assessing innovative therapies, such as qMRI or the use of biomarkers such as miRNAs. After diagnosis, researchers may face the main challenge of defining objective and quantitative markers that are not affected by inter-observer variability. Precise guidelines for the management and monitoring of the disease are also needed.

REFERENCES

1. Taverna S, Cammarata G, Colomba P, et al. Pompe disease: pathogenesis, molecular genetics and diagnosis. Aging (Albany NY). 2020;12(15):15856–15874. doi:10.18632/aging.103794
2. Peruzzo P, Pavan E, Dardis A. Molecular genetics of Pompe disease: a comprehensive overview. Ann Transl Med. 2019;7(13):278. doi:10.21037/atm.2019.04.13
3. Stevens D, Milani-Nejad S, Mozaffar T. Pompe Disease: a Clinical, Diagnostic, and Therapeutic Overview. Curr Treat Options Neurol. 2022;24(11):573–588. doi:10.1007/s11940-022-00736-1 22
4. Hahn A, Schänzer A. Long-term outcome and unmet needs in infantile-onset Pompe disease. Ann Transl Med. 2019;7(13):283. doi:10.21037/atm.2019.04.70
5. Labella B, Cotti Piccinelli S, Risi B, et al. A Comprehensive Update on Late-Onset Pompe Disease. Biomolecules. 2023;13(9):1279. Published 2023 Aug 22. doi:10.3390/biom13091279
6. Prakash S, Penn JD, Jackson KE, Dean LW. Newborn screening for Pompe disease: Parental experiences and follow-up care for a late-onset diagnosis. J Genet Couns. 2022;31(6):1404–1420. doi:10.1002/jgc4.1615
7. El Haddad L, Khan M, Soufny R, et al. Monitoring and Management of Respiratory Function in Pompe Disease: Current Perspectives. Ther Clin Risk Manag. 2023;19:713–729. Published 2023 Sep 1. doi:10.2147/TCRM.S362871
8. Herbert M, Case LE, Rairikar M, et al. Early-onset of symptoms and clinical course of Pompe disease associated with the c.-32–13 T > G variant. Mol Genet Metab. 2019;126(2):106–116. doi:10.1016/j.ymgme.2018.08.009
9. Nancy Leslie, Laurie Bailey. Pompe Disease. GeneReviews®, edited by Adam MP, et al. University of Washington, Seattle, 31 August 2007.
10. Pfrimmer C, Smitka M, Muschol N, et al. Long-Term Outcome of Infantile Onset Pompe Disease Patients Treated with Enzyme Replacement Therapy – Data from a German-Austrian Cohort. J Neuromuscul Dis. 2024;11(1):167–177. doi:10.3233/JND-230164
11. Kenney-Jung D, Korlimarla A, Spiridigliozzi GA, et al. Severe CNS involvement in a subset of long-term treated children with infantile-onset Pompe disease. Mol Genet Metab. 2024;141(2):108119. doi:10.1016/j.ymgme.2023.108119
12. Toscano A, Rodolico C, Musumeci O. Multisystem late onset Pompe disease (LOPD): an update on clinical aspects. Ann Transl Med. 2019;7(13):284. doi:10.21037/atm.2019.07.24
13. Jastrzębska A, Kostera-Pruszczyk A. Multisystem presentation of Late Onset Pompe Disease: what every consulting neurologist should know. Neurol Neurochir Pol. 2023;57(2):143–150. doi:10.5603/PJNNS.a2022.0075
14. Pompe disease GAA variant database; https://www.pompevariantdatabase.nl/pompe_mutations_list.php?orderby=aMut_ID1 (access: 2024.02.15)
15. Kroos M, Pomponio RJ, van Vliet L, et al. Update of the Pompe disease mutation database with 107 sequence variants and a format for severity rating. Hum Mutat. 2008;29(6):E13-E26. doi:10.1002/humu.20745
16. de Faria DOS, 't Groen SLMI, Hoogeveen-Westerveld M, et al. Update of the Pompe variant database for the prediction of clinical phenotypes: Novel disease-associated variants, common sequence variants, and results from newborn screening. Hum Mutat. 2021;42(2):119–134. doi:10.1002/humu.24148
17. Momosaki K, Kido J, Yoshida S, et al. Newborn screening for Pompe disease in Japan: report and literature review of mutations in the GAA gene in Japanese and Asian patients. J Hum Genet. 2019;64(8):741–755. doi:10.1038/s10038-019-0603-7
18. Kuperus E, van der Meijden JC, In 't Groen SLM, et al. The ACE I/D polymorphism does not explain heterogeneity of natural course and

response to enzyme replacement therapy in Pompe disease. PLoS One. 2018;13(12):e0208854. Published 2018 Dec 7. doi:10.1371/journal.pone.0208854
19. van der Ploeg AT, Kruijshaar ME, Toscano A, et al. European consensus for starting and stopping enzyme replacement therapy in adult patients with Pompe disease: a 10-year experience. Eur J Neurol. 2017;24(6):768-e31. doi:10.1111/ene.13285
20. Chien YH, Hwu WL, Lee NC. Newborn screening: Taiwanese experience. Ann Transl Med. 2019;7(13):281. doi:10.21037/atm.2019.05.47
21. Sawada T, Kido J, Nakamura K. Newborn Screening for Pompe Disease. Int J Neonatal Screen. 2020;6(2):31. Published 2020 Apr 5. doi:10.3390/ijns6020031
22. Feeney EJ, Austin S, Chien YH, et al. The value of muscle biopsies in Pompe disease: identifying lipofuscin inclusions in juvenile- and adult-onset patients. Acta Neuropathol Commun. 2014;2:2. Published 2014 Jan 2. doi:10.1186/2051-5960-2-2
23. Manganelli F, Ruggiero L. Clinical features of Pompe disease. Acta Myol. 2013;32(2):82–84.
24. Reynolds TM, Tylee K, Booth K, Wierzbicki AS. PATHFINDER Project Collaboration group; Collaborators and research nurses as listed below. Identification of patients with Pompé disease using routine pathology results: PATHFINDER (creatine kinase) study. J Clin Pathol. 2019;72(12):805–809. doi:10.1136/jclinpath-2019-205711
25. Willis TA, Hollingsworth KG, Coombs A, et al. Quantitative muscle MRI as an assessment tool for monitoring disease progression in LGMD2I: a multicentre longitudinal study. PLoS One. 2013;8(8):e70993. Published 2013 Aug 14. doi:10.1371/journal.pone.0070993
26. Seiler A, Nöth U, Hok P, et al. Multiparametric Quantitative MRI in Neurological Diseases. Front Neurol. 2021;12:640239. Published 2021 Mar 8. doi:10.3389/fneur.2021.640239
27. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. Front Endocrinol (Lausanne). 2018;9:402. Published 2018 Aug 3. doi:10.3389/fendo.2018.00402. 27
28. Tarallo A, Carissimo A, Gatto F, et al. microRNAs as biomarkers in Pompe disease. Genet Med. 2019;21(3):591–600. doi:10.1038/s41436-018-0103-8
29. Ditters IAM, Huidekoper HH, Kruijshaar ME, et al. Effect of alglucosidase alfa dosage on survival and walking ability in patients with classic infantile Pompe disease: a multicentre observational cohort study from the European Pompe Consortium. Lancet Child Adolesc Health. 2022;6(1):28–37. doi:10.1016/S2352-4642(21)00308-4
30. Bolano-Diaz C, Diaz-Manera J. Therapeutic Options for the Management of Pompe Disease: Current Challenges and Clinical Evidence in Therapeutics and Clinical Risk Management. Ther Clin Risk Manag. 2022;18:1099–1115. Published 2022 Dec 13. doi:10.2147/TCRM.S334232
31. Kishnani PS, Nicolino M, Voit T, et al. Chinese hamster ovary cell-derived recombinant human acid alpha-glucosidase in infantile-onset Pompe disease. J Pediatr. 2006;149(1):89–97. doi:10.1016/j.jpeds.2006.02.035
32. Chien YH, Tsai WH, Chang CL, et al. Earlier and higher dosing of alglucosidase alfa improve outcomes in patients with infantile-onset Pompe disease: Evidence from real-world experiences. Mol Genet Metab Rep. 2020;23:100591. Published 2020 Apr 29. doi:10.1016/j.ymgmr.2020.100591
33. Khan AA, Case LE, Herbert M, et al. Higher dosing of alglucosidase alfa improves outcomes in children with Pompe disease: a clinical study and review of the literature. Genet Med. 2020;22(5):898–907. doi:10.1038/s41436-019-0738-0
34. Ditters IAM, van Kooten HA, van der Beek NAME, van der Ploeg AT, Huidekoper HH, van den Hout JMP. Are Anti-rhGAA Antibodies a Determinant of Treatment Outcome in Adults with Late-Onset Pompe Disease? A Systematic Review. Biomolecules. 2023;13(9):1414. Published 2023 Sep 19. doi:10.3390/biom13091414
35. Li C, Desai AK, Gupta P, et al. Transforming the clinical outcome in CRIM-negative infantile Pompe disease identified via newborn screening: the benefits of early treatment with enzyme replacement therapy and immune tolerance induction. Genet Med. 2021;23(5):845–855. doi:10.1038/s41436-020-01080-y
36. Yang CF, Yang CC, Liao HC, et al. Very Early Treatment for Infantile-Onset Pompe Disease Contributes to Better Outcomes. J Pediatr. 2016;169:174–80.e1. doi:10.1016/j.jpeds.2015.10.078
37. Unnisa Z, Yoon JK, Schindler JW, Mason C, van Til NP. Gene Therapy Developments for Pompe Disease. Biomedicines. 2022;10(2):302. Published 2022 Jan 28. doi:10.3390/biomedicines10020302
38. U.S National Library of Medicine, database of privately and publicly funded clinical studies; https://clinicaltrials.gov/ (access: 2024.01.29)
39. Diaz-Manera J, Kishnani PS, Kushlaf H, et al. Safety and efficacy of avalglucosidase alfa versus alglucosidase alfa in patients with late-onset Pompe disease (COMET): a phase 3, randomised, multicentre trial [published correction appears in Lancet Neurol. 2022 Apr;21(4):e4]. Lancet Neurol. 2021;20(12):1012–1026. doi:10.1016/S1474-4422(21)00241-6
40. Blair HA. Cipaglucosidase Alfa: First Approval. Drugs. 2023;83(8):739–745. doi:10.1007/s40265-023-01886-5
41. Schoser B, Roberts M, Byrne BJ, et al. Safety and efficacy of cipaglucosidase alfa plus miglustat versus alglucosidase alfa plus placebo in late-onset Pompe disease (PROPEL): an international, randomised, double-blind, parallel-group, phase 3 trial [published correction appears in Lancet Neurol. 2023 Oct;22(10):e11]. Lancet Neurol. 2021;20(12):1027–1037. doi:10.1016/S1474-4422(21)00331-8
42. Mingozzi F, High KA. Overcoming the Host Immune Response to Adeno-Associated Virus Gene Delivery Vectors: The Race Between Clearance, Tolerance, Neutralization, and Escape. Annu Rev Virol. 2017;4(1):511–534. doi:10.1146/annurev-virology-101416-041936
43. Smith BK, Martin AD, Lawson LA, et al. Inspiratory muscle conditioning exercise and diaphragm gene therapy in Pompe disease: Clinical evidence of respiratory plasticity. Exp Neurol. 2017;287(Pt 2):216–224. doi:10.1016/j.expneurol.2016.07.013
44. Eggers M, Vannoy CH, Huang J, et al. Muscle-directed gene therapy corrects Pompe disease and uncovers species-specific GAA immunogenicity. EMBO Mol Med. 2022;14(1):e13968. doi:10.15252/emmm.202113968
45. Hordeaux J, Dubreil L, Robveille C, et al. Long-term neurologic and cardiac correction by intrathecal gene therapy in Pompe disease. Acta Neuropathol Commun. 2017;5(1):66. Published 2017 Sep 6. doi:10.1186/s40478-017-0464-2
46. Keeler AM, Zieger M, Todeasa SH, et al. Systemic Delivery of AAVB1-GAA Clears Glycogen and Prolongs Survival in a Mouse Model of Pompe Disease. Hum Gene Ther. 2019;30(1):57–68. doi:10.1089/hum.2018.016
47. Hordeaux J, Wang Q, Katz N, Buza EL, Bell P, Wilson JM. The Neurotropic Properties of AAV-PHP.B Are Limited to C57BL/6J Mice. Mol Ther. 2018;26(3):664–668. doi:10.1016/j.ymthe.2018.01.018
48. Stok M, de Boer H, Huston MW, et al. Lentiviral Hematopoietic Stem Cell Gene Therapy Corrects Murine Pompe Disease. Mol Ther Methods Clin Dev. 2020;17:1014–1025. Published 2020 May 4. doi:10.1016/j.omtm.2020.04.023
49. van der Wal E, Bergsma AJ, Pijnenburg JM, van der Ploeg AT, Pijnappel WWMP. Antisense Oligonucleotides Promote Exon Inclusion and Correct the Common c.-32–13T>G GAA Splicing Variant in Pompe Disease. Mol Ther Nucleic Acids. 2017;7:90–100. doi:10.1016/j.omtn.2017.03.001
50. Muñoz S, Bertolin J, Jimenez V, et al. Treatment of infantile-onset Pompe disease in a rat model with muscle-directed AAV gene therapy. Mol Metab. 2024;81:101899. doi:10.1016/j.molmet.2024.101899