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Review

Advances in Lafora Disease and Related Polyglucosan Disorders Therapy: From Drug Repurposing to Gene-Based Approaches

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Abstract

Lafora disease (LD) is a fatal progressive myoclonus epilepsy caused by biallelic pathogenic variants in *EPM2A* or *NHLRC1*, resulting in the accumulation of poorly branched, insoluble glycogen aggregates known as Lafora bodies (LBs). Over the past decade, advances in understanding LD pathogenesis have progressively redefined the role of abnormal glycogen metabolism and highlighted the central contribution of polyglucosan accumulation to disease pathogenesis. These mechanistic insights have substantially changed the therapeutic perspective, shifting the focus from symptomatic management toward interventions aimed at modifying the underlying disease processes. This review provides an updated overview of therapeutic advances reported between 2022 and 2026, with a primary focus on LD while highlighting selected developments in the related polyglucosan disorders Adult Polyglucosan Body Disease and Glycogen Storage Disease type IV. Despite major challenges remaining, recent progress provides a rapidly evolving framework for the development of disease-modifying treatments for LD and offers relevant insights into the broader therapeutic potential and limitations of targeting pathological glycogen in polyglucosan disorders.

Keywords: Lafora disease; glycogen metabolism; polyglucosan; antisense oligonucleotides; neurodegeneration; APBD; GSD IV; metabolic therapy

1. Introduction

Glycogen storage diseases (GSDs) involving the central nervous system (CNS) comprise a heterogeneous group of inherited metabolic disorders in which alterations in glycogen synthesis, structure, or turnover can lead to the accumulation of poorly branched and insoluble glycogen-like polymers known as polyglucosans [1,2]. Among these disorders, Lafora disease (LD) is recognized as the most severe, presenting as a progressive myoclonus epilepsy with onset typically during childhood or adolescence and rapid neurological deterioration, including seizures, myoclonus, cognitive decline, and progressive loss of motor function [3–5]. Other clinically relevant polyglucosan disorders include Adult Polyglucosan Body Disease (APBD), a predominantly late-onset neurological disorder, and Glycogen Storage Disease Type IV (GSD IV or Andersen disease), which encompasses a heterogeneous spectrum of hepatic, neuromuscular, and neurological manifestations [6,7]. Despite their distinct genetic backgrounds, age at onset, and clinical manifestations, LD, APBD, and GSD IV converge on a common pathological feature: the progressive accumulation of structurally abnormal glycogen in the form of polyglucosans, ultimately leading to cellular and tissue dysfunction. From a molecular point of view, LD results from pathogenic variants in *EPM2A* or *NHLRC1* (also known as *EPM2B*), whereas APBD and GSD IV are associated with pathogenic variants in *GBE1* [1,8].

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This shared metabolic vulnerability is particularly relevant from a therapeutic perspective. Unlike neurodegenerative diseases primarily associated with protein aggregation, GSDs involve the accumulation of an abnormal carbohydrate polymer whose synthesis, structure, and turnover may potentially be pharmacologically or genetically manipulated. Recent advances in glycogen metabolism have opened new opportunities for mechanism-based interventions, ranging from small-molecule and drug-repurposing approaches to modulation of glycogen synthesis and proteostasis, antisense oligonucleotides (ASOs) and other RNA-based therapeutics, and gene-based strategies. Particular attention has been directed toward targeting pathological glycogen accumulation, offering new therapeutic opportunities while raising important translational challenges for interventions within the CNS.

Literature Search Strategy and Scope

A literature search was conducted using PubMed, Scopus, and Web of Science, focusing on publications from 2022 to 2026. Search terms included "Lafora disease", "polyglucosan body disease", "glycogen storage disease type IV", "GYS1", "drug repurposing", "small molecules", "antisense oligonucleotides", and "gene therapy". Original research articles, clinical studies, and relevant reviews addressing disease mechanisms and emerging therapeutic strategies were considered, while duplicate publications and studies unrelated to the scope of this review were excluded. Earlier landmark studies were also included when necessary to provide essential mechanistic and historical context.

As this is a narrative rather than a systematic review, the literature was selected based on its scientific relevance to the topics discussed, without applying formal systematic-review procedures.

Accordingly, this review provides an updated overview of emerging therapeutic strategies for LD reported between 2022 and 2026, primarily focusing on approaches targeting polyglucosan formation, accumulation, and persistence, while also highlighting relevant therapeutic advances in the related disorders APBD and GSD IV, as summarized in Table 1. Where applicable, half-maximal inhibitory concentrations (IC_{50}) are reported.

2. Lafora Disease: Physiopathology and Challenges

The pathophysiology of LD is closely linked to the disruption of glycogen homeostasis in the central nervous system. Brain glycogen is a dynamic metabolic reserve involved not only in energy homeostasis but also in neuronal activity, neurotransmission, synaptic plasticity, and cellular responses to metabolic stress [9]. Its structure and turnover therefore require tight regulation of glycogen synthesis, branching, degradation, and quality-control mechanisms. Within this system, the laforin–malin complex plays a critical role in maintaining glycogen structural integrity. Laforin, encoded by *EPM2A*, is a glycogen-binding dual-specificity phosphatase involved in the removal of covalently bound phosphate from glycogen [10,11], whereas malin, encoded by *NHLRC1*, is an E3 ubiquitin ligase that functionally interacts with laforin [12–14]. Loss of a functional laforin–malin complex progressively alters glycogen architecture, leading to the formation of Lafora bodies (LBs), the pathological hallmark of LD. Compared with physiological glycogen, LBs are poorly branched, contain longer glucan chains, and display markedly reduced solubility, features that classify them as polyglucosans [15,16]. Glycogen from LD patients and experimental models is also consistently hyperphosphorylated [17,18]. However, hyperphosphorylation alone does not fully explain disease pathogenesis, as phosphatase-deficient laforin can prevent LB formation under specific experimental conditions [19]. These observations suggest that laforin and malin cooperate in glycogen quality control through mechanisms extending beyond the phosphatase activity of laforin [15,16,19]. Interestingly, only a subset of glycogen molecules

appears to acquire the structural abnormalities that promote precipitation, suggesting that the laforin–malin complex may preferentially act on glycogen molecules at risk of losing their normal architecture and solubility [20].

The central pathogenic role of abnormal glycogen accumulation is strongly supported by experimental evidence. Genetic or pharmacological reduction in glycogen synthesis, through *Gys1* deletion, reduction in its regulatory protein PTG (protein targeting to glycogen), or antisense-mediated *Gys1* downregulation, markedly prevents LB formation, reduces neuroinflammation, and improves neurological abnormalities in LD mouse models [21,22].

Despite these promising advances, most therapeutic strategies discussed in this review remain at the preclinical stage, with efficacy demonstrated primarily in cellular and animal models. Human evidence is currently limited to preliminary observations involving metformin in LD and **GHF-201** in a small number of patients with APBD. Therefore, although these approaches provide valuable proof-of-concept, their clinical efficacy, safety, and long-term benefits remain to be established through adequately designed clinical studies.

Neuroinflammation also appears to contribute substantially to disease progression. *Epm2a−/−* and *Epm2b−/−* mouse models display early reactive astrogliosis and microgliosis that progressively increase with age and precede extensive neuronal loss. Transcriptomic analyses similarly reveal prominent activation of inflammatory pathways [23]. Activation of Tumor Necrosis Factor (TNF) and IL-6/JAK2 (Interleukin-6/Janus Kinase 2) signaling, together with increased inflammatory mediators and peripheral immune-cell infiltration, further supports an active contribution of neuroinflammation to LD progression [24]. Importantly, glial dysfunction may also contribute directly to neuronal hyperexcitability. Alterations in glutamatergic and GABAergic signaling have been identified in reactive astrocytes and microglia, potentially shifting neuronal networks toward increased excitation and reduced inhibitory tone [25].

Synaptic dysfunction is consequently emerging as another early component of LD pathophysiology. Loss of the laforin–malin complex impairs trafficking and membrane localization of the astrocytic glutamate transporter GLT-1 (EAAT2), reducing glutamate uptake and promoting excessive glutamatergic signaling [26,27]. In parallel, degeneration of cortical GABAergic interneurons and alterations in synaptic proteins further compromise inhibitory neurotransmission [28]. Consistent with these observations, electrophysiological studies in the *Epm2aR240X* knock-in model demonstrated hippocampal hyperexcitability and impaired synaptic plasticity before widespread neurodegeneration [29]. Thus, neuronal network dysfunction appears to represent an early pathogenic event rather than merely a consequence of neuronal loss.

Alterations in autophagy and proteostasis may further amplify cellular dysfunction. Reduced autophagic activity and defects in endosomal–lysosomal pathways have been reported in LD cellular and animal models [30,31]. Given the E3 ubiquitin ligase activity of malin, impaired protein quality control has also been proposed as a contributor to pathogenesis, although the physiological relevance of several reported malin substrates remains uncertain [32]. Likewise, whether autophagic dysfunction represents a primary consequence of laforin–malin deficiency or develops secondarily to abnormal glycogen accumulation remains unresolved. This distinction is particularly relevant in the context of glycophagy, the selective lysosomal degradation of glycogen [33]. Mature polyglucosan inclusions appear to be poorly susceptible to autophagic clearance, suggesting that enhancing glycophagy may have limited efficacy once LBs are established [8,34] (Figure 1).

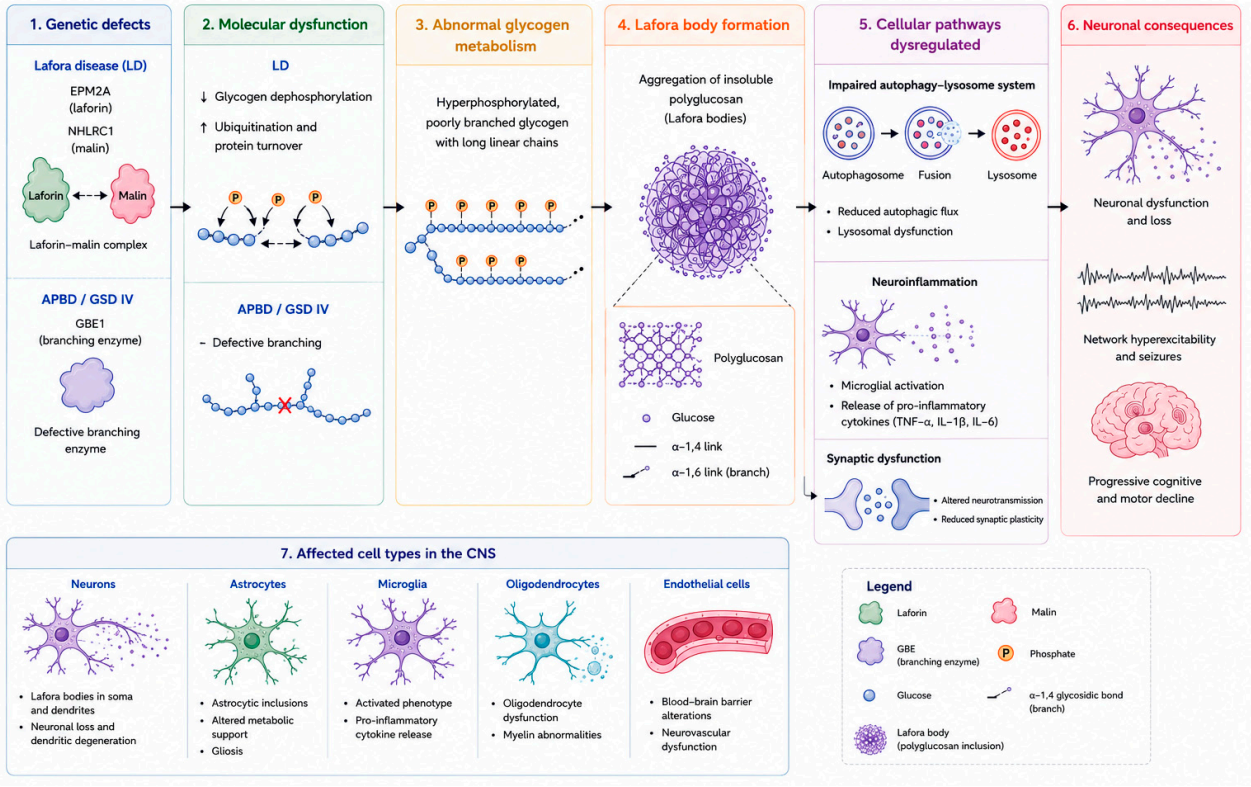


Figure 1. Pathophysiology of GSD.

The growing understanding of LD pathophysiology has progressively identified pathological glycogen accumulation as the main therapeutic target, with reduction in glycogen synthesis, particularly through GYS1 modulation, emerging as a promising strategy. At the same time, restoration of the defective laforin-malin pathway and approaches aimed at facilitating the clearance of accumulated polyglucosans represent additional opportunities for disease modification, while downstream processes such as neuroinflammation and impaired proteostasis may provide complementary targets. However, translating these approaches into effective therapies remains challenging, particularly because of the limited accessibility of the CNS, the difficulty of removing established LBs, and the progressive nature of neuronal dysfunction. These considerations make the timing of intervention particularly relevant and highlight the need to determine whether targeting glycogen accumulation alone will be sufficient once downstream pathological processes have become established.

3. Drug Repositioning to Target Polyglucosan Disorders

Because LD, APBD, and GSD IV are ultra-rare diseases, de novo drug development is associated with substantial financial and regulatory challenges. Repurposing approved compounds with established pharmacokinetic and toxicological profiles offers the possibility of substantially accelerating clinical translation. Compounds targeting oxidative stress, mitochondrial dysfunction, neuroinflammation, and lysosomal activity have been proposed as candidate adjunctive therapies, although robust preclinical validation remains limited [1].

3.1. Metformin

One of the best characterized pharmacological candidates is metformin (*N*,*N*-Dimethylimidodicarbonimidic diamide) (Figure 2), a biguanide widely prescribed for type 2 diabetes mellitus. Metformin activates AMP-activated protein kinase (AMPK), a central metabolic sensor that suppresses anabolic pathways while promoting catabolic processes,

including fatty acid oxidation and, under selected conditions, autophagy [35,36]. Beyond its metabolic actions, metformin has demonstrated anti-inflammatory, antioxidant, antiseizure properties in mice models of induced epilepsy using the chemoconvulsant pentylenetetrazole (PTZ) and neuroprotective properties in several neurodegenerative disease models [37]. These pleiotropic effects prompted investigation of its therapeutic potential in LD, where neuroinflammation and oxidative stress substantially contribute to disease progression [38].

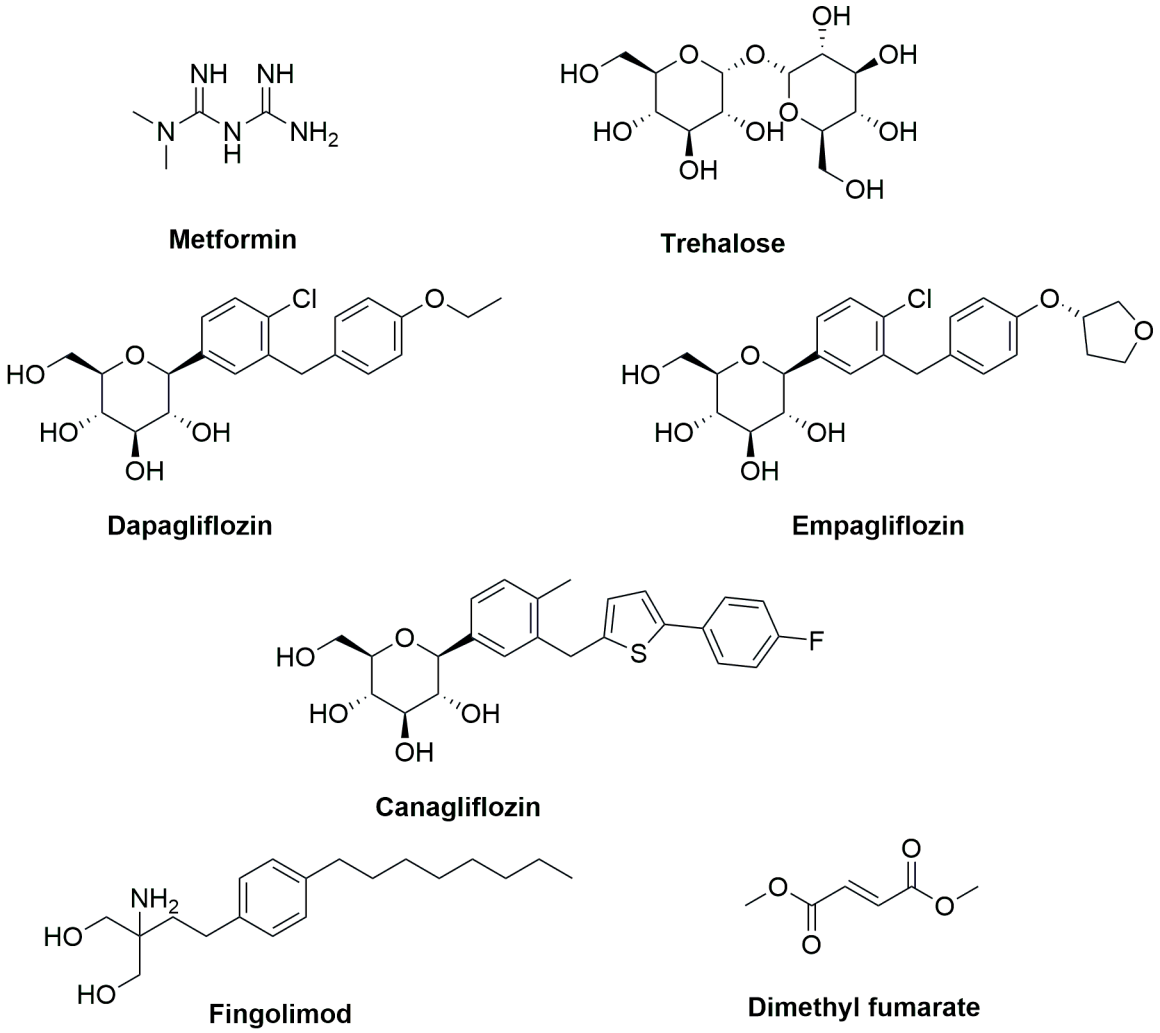


Figure 2. Chemical structures of clinically approved drugs investigated for repurposing and trehalose, a dietary disaccharide explored as a potential therapeutic agent for LD.

Despite metformin being already recognized as an orphan drug for the treatment of LD by the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) in 2016 and 2017, respectively, further studies were conducted [39].

In detail, a more recent study investigated the therapeutic potential of metformin in improving neurological outcomes in LD using both *Epm2a*−/− and *Epm2b*−/− mouse models and clinical data from 18 patients. Mice received either early treatment (from conception to adulthood; E-MET) or late treatment (initiated at 10 months of age; L-MET), and neurological, behavioral, and histopathological outcomes were evaluated. In parallel, disease progression in metformin-treated and untreated patients was assessed using the Lafora Epilepsy Severity Scale (LESS).

Early metformin treatment was significantly more effective than late treatment, reducing seizure susceptibility, LB accumulation, neurodegeneration, astrogliosis, and motor and

cognitive deficits. In *Epm2b−/−* mice, early treatment completely prevented PTZ-induced seizures. Clinically, metformin-treated patients exhibited slower disease progression, with lower LESS scores over a 24-month follow-up, while maintaining better language and daily living abilities. The treatment was generally well tolerated, with only mild adverse effects. Overall, the findings suggest that early metformin administration provides greater neuroprotective benefits than delayed treatment and may represent a safe therapeutic strategy for LD. However, larger, controlled clinical trials are required to confirm its long-term efficacy and support its use in presymptomatic or early-stage patients [29].

3.2. Trehalose

Another promising class of compounds comprises autophagy-enhancing agents, particularly trehalose (Figure 2), a naturally occurring disaccharide extensively investigated in protein aggregation disorders. Trehalose promotes autophagic activity through largely mTOR-independent mechanisms, stabilizes protein conformation, and reduces intracellular aggregate formation. Experimental studies across several neurodegenerative diseases have consistently demonstrated improvements in proteostasis and attenuation of neuroinflammatory responses. To address this objective, Della Vecchia and collaborators developed and characterized an *Epm2a-/-* knock-out zebrafish model of LD to investigate the consequences of laforin loss of function on cell metabolism and early neurodevelopment, and to identify a reliable and versatile model for carrying out new drug screenings. In detail, in this study the therapeutic potential of trehalose was evaluated, finding that treatment significantly improved locomotor performance reduced neuronal apoptosis, and attenuated glycogen accumulation and neuroinflammation in mutant zebrafish. These findings demonstrate that the zebrafish model successfully recapitulates key pathological features of LD and represents a rapid, cost-effective platform for preclinical drug screening. Moreover, the study provides further evidence supporting trehalose, administered in the early stages, as a potential disease-modifying therapy, particularly through its neuroprotective effects and its ability to mitigate glycogen-related pathology [40].

3.3. Sodium–Glucose Cotransporter-2 Inhibitors

Sodium–glucose cotransporter-2 (SGLT2) inhibitors represent attractive candidates for the treatment of LD because of their ability to modulate systemic and cellular glucose metabolism. By promoting urinary glucose excretion, these agents reduce glucose availability, potentially limiting glycogen synthesis and the formation of poorly branched glycogen that gives rise to LB. In addition, SGLT2 inhibitors activate energy-sensing pathways, including AMPK, while suppressing mTOR signaling, thereby enhancing autophagy and facilitating the clearance of abnormal glycogen aggregates. They have also been shown to improve mitochondrial function, reduce oxidative stress and neuroinflammation, promote metabolic flexibility through increased ketone body utilization, and exert neuroprotective effects in several neurological disease models. Collectively, these pleiotropic actions provide a strong biological rationale for repurposing SGLT2 inhibitors as potential disease-modifying therapies for LD, although their efficacy remains to be demonstrated in dedicated preclinical and clinical studies [41]. To this purpose, Della Vecchia and collaborators, once assessed the effectiveness of the *Epm2a -/-* zebrafish model, investigated the efficacy of a series of SGLT2 inhibitors approved for use in type 2 diabetes mellitus, heart failure and chronic kidney disease, such as dapagliflozin (50 μM), canagliflozin (2.5 μM) and empagliflozin (200 μM) (Figure 2). What was observed is a beneficial role played by dapagliflozin in the larvae model, such it improves neuronal excitability, locomotor activity, and seizures; it slightly reduces total larvae glycogen content, and inflammatory and oxidative stress markers; and improves autophagic homeostasis and normalizes lysosomal markers. Despite

the encouraging results, further studies are required to investigate the therapeutic potential and mechanisms of action of dapagliflozin in LD [42]. An ongoing study protocol proposes an integrated in silico and in vitro evaluation of empagliflozin to identify its molecular targets and validate its effects on glycogen metabolism in LD. These studies are expected to provide mechanistic insights and support the potential repurposing of SGLT2 inhibitors as disease-modifying therapies [43].

3.4. Fingolimod and Dimethyl Fumarate

Rubio et al. described the beneficial effects of the oral daily administration of fingolimod (FGD) and dimethyl fumarate (DMF) in LD mouse models (Figure 2) [44]. The aim of this study was to prevent the infiltration of T-lymphocytes in the brain parenchyma of LD mice, which could aggravate the neuroinflammation scenario already present in LD patients. FGD has been approved for the treatment of multiple sclerosis by the FDA in 2010. From a CNS point of view, as an antagonist of S1PR1 signaling, FGD exerts broad neuroprotective and anti-inflammatory effects by preserving the blood–brain barrier (BBB) integrity, reducing glial activation and pro-inflammatory cytokine production, and promoting a shift toward an anti-inflammatory microglial phenotype. In addition, it protects neurons from excitotoxicity, enhances oligodendrocyte-mediated remyelination, and supports neurogenesis [45]. In LD mouse models, fingolimod reduced astrocyte activation, attenuated TNF and IL-6 inflammatory signaling, and decreased T-lymphocyte infiltration into the brain parenchyma.

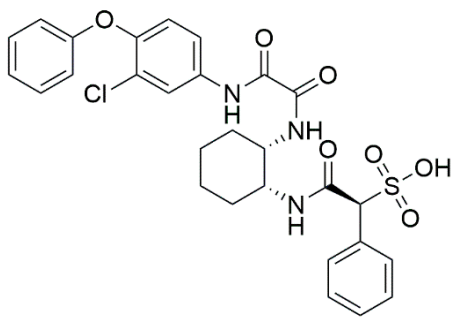
DMF, approved by the FDA in 2013 for the treatment of multiple sclerosis, exerts antioxidant and immunomodulatory effects primarily through *Nrf2* activation and *HCAR2* agonism, reducing microglial and astrocyte activation and limiting the production of pro-inflammatory mediators.

However, in LD models, although DMF attenuated glial activation, it had little effect on inflammatory signaling or T-cell infiltration, resulting in only limited improvements in behavioral outcomes [46,47]. FGD improved both behavioral and histopathological outcomes in *Epm2b−/−* mice, supporting its therapeutic potential in LD. The authors further suggest that FGD may be most effective as part of a drug repurposing combination strategy, where synergistic interactions with agents such as metformin, propranolol, or memantine could enhance disease modification [44].

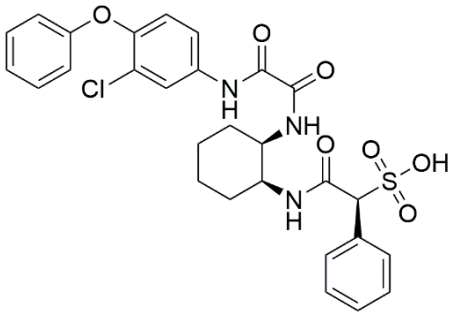
4. Synthetic Small Molecules

Small-molecule compounds remain highly attractive because of their relatively low production costs, oral bioavailability, established safety profiles, and easier clinical implementation. However, most compounds investigated to date exert indirect effects on glycogen metabolism or cellular homeostasis rather than correcting the underlying molecular defect [1].

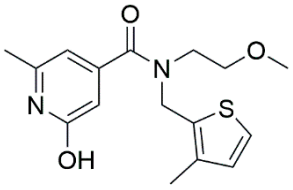
Lin et al. [48] identified, through fragment-based and scaffold-hopping approaches, sulfophenyl acetic amide (SPAA) as a novel nonhydrolyzable phosphotyrosine mimetic, highly potent and selective active site-directed laforin inhibitors. Among the series compound **9c** ((S)-2-((2-(2-((3-chloro-4-phenoxyphenyl)amino)-2-oxoacetamido)cyclohexyl)amino)-2-oxo-1-phenylethanesulfonic acid, a mixture of two cis-diastereomers) (Figure 3) displayed an IC_{50} value of 4.0 ± 0.3 nM, a Ki value of 1.9 ± 0.2 nM and more than 8300-fold preference for laforin, acting as reversible and competitive active site-directed inhibitor. Moreover, docking studies predicted that the derivative binds to the maltohexaose binding site of laforin (between its sulfonic acid moiety and the backbone amide hydrogens of laforin active site residues Ala268, Val270, Gly271, and Arg272; the residue Trp196, which participates in the π−π stacking interaction with the distal phenyl ring of compound **9c**, is unique to laforin and is absent in all other tested protein tyrosine phosphatases (PTP) enzymes) [48].



(S)-2-(((1R,2S)-2-(2-((3-chloro-4-phenoxyphenyl)amino)-2-oxoacetamido)cyclohexyl)amino)-2-oxo-1-phenylethane-1-sulfonic acid



(S)-2-(((1S,2R)-2-(2-((3-chloro-4-phenoxyphenyl)amino)-2-oxoacetamido)cyclohexyl)amino)-2-oxo-1-phenylethane-1-sulfonic acid



GHF-201

Figure 3. Chemical structures of the cis-diastereomers of compound **9c** and **GHF-201**.

Recently, **GHF-201** has emerged as a promising small-molecule candidate for APBD (Figure 3). Subsequently, a Phase I randomized, double-blind, placebo-controlled study has evaluated the safety, tolerability, and pharmacokinetics of **GHF-201** in healthy volunteers (EUCT 2024-519813-74-00), while preliminary compassionate-use data reported in 2026 in APBD derives a program involving only three patients observing a reduction in polyglucosan bodies (~81% in fibroblasts and 91% in lymphocytes) [49]. While these early findings are encouraging, the limited number of patients and the preliminary nature of the available data warrant caution. Further studies involving larger patient cohorts are needed to confirm the clinical efficacy and long-term safety of GHF-201 in APBD.

GHF-201, originally designated **144DG11**, was identified through phenotypic high-throughput screening of synthetic small molecules for their ability to reduce polyglucosan accumulation in APBD patient-derived fibroblasts [50]. Subsequent mechanistic studies identified the Lysosomal-Associated Membrane Protein 1 (LAMP1) as its molecular target. Interaction with LAMP1 enhances lysosomal acidification and autophagic flux, thereby promoting autolysosomal glycogen degradation [34]. The therapeutic potential of **GHF-201** has subsequently been extended to other glycogen storage disorders, with beneficial metabolic and functional effects also reported in experimental models of GSD III [51]. Notably, however, these effects do not appear to extend to LD. In *Epm2a*−/− mice, **GHF-201** failed to reduce polyglucosan bodies in the brain, heart, or skeletal muscle and did not attenuate the associated astrogliosis or microgliosis [8]. This differential response may reflect important differences in the mechanisms underlying polyglucosan accumulation and clearance in LD and APBD. Wu et al. proposed that glycogen dephosphorylation might be required for the autophagic degradation of accumulated polyglucosans, potentially explaining why GHF-201 fails to promote their clearance in laforin-deficient mice [8]. An additional possibility is that alterations in the autophagic machinery itself may limit the effectiveness of pharmacological autophagy enhancement in LD, although evidence for a primary autophagic defect remains inconclusive. These hypotheses require further investigation but suggest that the therapeutic response to autophagy-enhancing compounds may depend on disease-specific mechanisms beyond polyglucosan accumulation alone.

5. Antisense Oligonucleotides and Therapeutic Potential in Glycogen Storage Disorders

ASOs are short, synthetic single-stranded nucleic acids, typically 15–25 nucleotides in length, designed to selectively modulate gene expression through interaction with messenger RNA (mRNA) or precursor mRNA (pre-mRNA) [52,53]. In this context, LD has emerged as the prototype for ASO-based therapeutic development. Rather than correcting the causative mutations in *EPM2A* or *NHLRC1*, current therapeutic strategies exploit the observation that even modest reductions in GYS1 activity are sufficient to prevent the formation of pathogenic polyglucosan inclusions while preserving physiological glycogen metabolism [54,55].

To reduce glycogen synthesis, Nitschke et al. [54] designed and screened 300 ASOs targeting different regions of *Gys1* pre-mRNA, identifying a lead candidate capable of achieving approximately 75% *Gys1* mRNA knockdown in vitro, with an IC_{50} of 3.4 µM. The selected mouse-specific ASO sequence was 5′-CATGCTTCATTTCTTTATTG-3′ and it acts through an RNase H1-dependent mechanism, promoting degradation of *Gys1* mRNA [56]. Following intracerebroventricular (ICV) administration to LD mouse models (*Epm2a*−/− and *Epm2b*−/−), treatment reduced *Gys1* mRNA and protein levels by >80%, decreased brain glycogen content by >50% to values comparable to those of wild-type (WT) mice, and almost completely prevented LB formation when initiated at 1 month of age, corresponding to the onset of LB deposition. In contrast, when treatment was initiated after pathology had already developed (3 or 8 months of age), the ASO did not eliminate pre-existing LBs but effectively prevented their further accumulation. Therapeutic efficacy was greater when treatment was started earlier, continued for a longer period (3–12 months versus 3–6 months), or administered at a higher dose (500 µg versus 300 µg), demonstrating that even partial suppression of GYS1 is sufficient to markedly attenuate disease progression and supporting ASO-mediated gene silencing as a promising disease-modifying strategy for LD [54].

Subsequently, the same group extended this approach to RBCK1 deficiency. This deficiency causes amylopectinosis with fatal skeletal and cardiac myopathy (polyglucosan body myopathy 1), a rare glycogen storage disorder characterized by the accumulation of abnormal polyglucosan bodies resulting from dysregulated glycogen metabolism. In a newly generated *Rbck1*-deficient mouse model, genetic reduction in Gys1 expression prevented polyglucosan accumulation, identifying GYS1 as a potential therapeutic target. The previously developed Gys1-ASO was therefore evaluated by intracerebroventricular administration, resulting in an approximately 50% reduction in *Gys1* mRNA, a 57% decrease in brain glycogen content, and complete prevention of polyglucosan body formation throughout the central nervous system. These findings demonstrate that targeting a downstream pathogenic pathway, rather than the primary disease-causing gene, can effectively prevent disease pathology, providing strong proof-of-concept for ASO-mediated gene silencing in polyglucosan storage disorders [54].

More recently, Donohue et al. [55] further validated the therapeutic potential of Gys1-ASO (5′-CAT GCTTCATTTCTTTATTG-3′), in the *Epm2b*−/− (malin knockout) mouse model of LD. Mice received ICV injections of 300 µg ASO in 10 µL PBS at 4, 7, and 10 months of age. Consistent with previous findings, the ASO exhibited broad biodistribution throughout the brain and significantly reduced Gys1 expression across all examined brain regions. In addition, a decrease in GYS1 protein levels was observed, as well as a reduction in glycogen accumulation and LB burden. Particularly in the hippocampus and cerebellum, where pathology is most prominent [55].

Importantly, ASO treatment also significantly reduced epileptiform discharges in the malin knockout model, supporting the hypothesis that limiting glycogen aggregation into

LBs reduces neuronal hyperexcitability. Although treatment did not significantly decrease neuroinflammatory markers, this was likely attributable to the relatively late initiation of therapy, when neuroinflammation was already established. Collectively, these findings further support GYS1 as a robust therapeutic target and indicate that ASO-mediated reduction in glycogen synthesis has the potential not only to limit pathological polyglucosan accumulation but also to ameliorate the neurological manifestations of LD, including progressive seizures and neurodegeneration [55].

The success of GYS1-targeting ASOs in preclinical LD models has also generated considerable interest in other polyglucosan disorders, such as APBD [57].

Although APBD differs genetically from LD, both disorders share a common pathological hallmark: the progressive accumulation of insoluble polyglucosan bodies resulting from abnormal glycogen structure [6,58,59]. This convergence in disease mechanisms has led to the hypothesis that modulation of glycogen synthesis, rather than correction of the primary genetic defect, may represent an effective therapeutic approach across multiple polyglucosan disorders [22,60].

A distinct ASO-based therapeutic strategy has recently been developed for APBD, aiming to correct the primary molecular defect rather than modulating glycogen synthesis [58,61]. Thomas et al. [57] investigated the recurrent deep intronic *GBE1* variant c.2053-3358_2053-3350delinsTGTTTTTTACATTACAGGT, demonstrating through long-read sequencing that it generates two aberrantly spliced transcripts containing a pseudoexon, both of which undergo nonsense-mediated decay. To restore physiological splicing, the authors designed and screened 77 splice-switching ASOs, identifying 16 lead candidates that efficiently redirected splicing toward the canonical *GBE1* transcript in a concentration-dependent manner. Treatment of patient-derived fibroblasts significantly increased correctly spliced *GBE1* mRNA and produced at least a 3.4-fold increase in *GBE1* protein levels for 14 of the lead ASOs. Functional analyses further demonstrated that five of the eight most effective ASOs significantly restored glycogen branching enzyme activity, increasing it from a baseline of 0.41-fold in untreated patient cells to an average of 0.75-fold of healthy control levels (range 0.67 to 0.83-fold), with GBE1 protein levels strongly correlating with enzymatic activity ($R^2 = 0.8$). These findings provide the first proof-of-concept that splice-switching ASOs can directly rescue both the molecular defect and enzymatic function in APBD, highlighting a precision medicine approach that complements GYS1-targeting therapies by restoring the activity of the disease-causing gene rather than reducing glycogen synthesis [57].

Despite these encouraging preclinical findings, several challenges remain for the clinical translation of ASO therapy in APBD. Unlike LD, APBD is typically diagnosed after years of progressive polyglucosan accumulation, emphasizing the need for earlier diagnosis and reliable biomarkers [1,6]. In addition, because APBD affects both the central and peripheral nervous systems, broader tissue biodistribution will be required to achieve optimal therapeutic efficacy. Finally, whether glycogen reduction alone can modify disease progression in patients with established neurological impairment remains to be determined [1,55].

More broadly, the clinical translation of ASO-based therapies faces several challenges, particularly regarding CNS delivery, tissue distribution, and long-term safety. Due to their limited ability to cross the BBB, ASOs generally require direct administration into the cerebrospinal fluid to achieve therapeutic concentrations within the CNS [53]. Although intrathecal administration provides a promising strategy to overcome this limitation, ensuring adequate and homogeneous distribution across different brain regions and relevant cellular populations remains a critical consideration, as ASO uptake and tissue exposure may vary considerably [62]. Furthermore, the prolonged tissue persistence of ASOs, while potentially beneficial for sustained therapeutic activity, requires careful evaluation of their

pharmacokinetic and safety profiles. Repeated administration may be necessary to maintain therapeutic effects, raising additional concerns regarding cumulative exposure, inflammatory responses, and long-term tolerability [63]. These aspects highlight the importance of comprehensive preclinical safety assessments and appropriate dosing strategies before clinical translation. Specifically, for GYS1-targeting ASOs, determining the optimal degree and duration of enzyme inhibition will be particularly important to achieve a therapeutically meaningful reduction in pathological glycogen accumulation without excessively interfering with physiological glycogen metabolism.

6. Gene Replacement Therapy

Gene replacement therapy has emerged as one of the most promising precision medicine strategies for monogenic neurological disorders and is increasingly being explored for polyglucosan diseases, particularly LD. Unlike substrate-reduction approaches based on glycogen synthase inhibition, gene replacement aims to restore the physiological function of the defective protein itself [64,65]. In LD, this involves the delivery of functional *EPM2A* or *NHLRC1* genes, thereby addressing the primary molecular defect responsible for disease pathogenesis rather than downstream metabolic consequences. This strategy has gained considerable momentum following the success of adeno-associated virus (AAV)-mediated therapies for several inherited neurological diseases, including spinal muscular atrophy and aromatic L-amino acid decarboxylase deficiency, demonstrating the feasibility of long-term transgene expression within the CNS [66,67].

The biological rationale for gene replacement in LD is particularly compelling because both causative genes encode relatively small proteins, whose coding sequences are well below the approximately 4.7 kb packaging capacity of recombinant AAV vectors [68]. Consequently, *EPM2A* or *NHLRC1* can be efficiently incorporated into single AAV constructs without the need for dual-vector systems, a major advantage over larger disease genes [69]. Restoration of laforin or malin expression is expected to re-establish the physiological glycogen quality-control complex, normalize glycogen architecture, reduce glycogen hyperphosphorylation, and ultimately prevent the formation of LBs. Earlier mechanistic studies established this concept and identified widespread CNS transduction as the principal challenge for successful clinical translation [70].

For several years, gene replacement remained largely theoretical because efficient CNS-wide delivery represented a major technical limitation. The rapid development of engineered AAV serotypes, including AAV9 and next-generation neurotropic capsids with enhanced CNS tropism following intrathecal or intracerebroventricular administration, has substantially improved the feasibility of gene replacement for neurological disorders [68,71].

In 2024, Zafra-Puerta et al. [72] evaluated the therapeutic potential of ICV administration of alglucosidase alfa in both *Epm2a−/−* knockout and *Epm2aR240X* knock-in mouse models of LD, using either a single injection or continuous infusion over two weeks. Neither treatment regimen reduced the number of LBs, nor did it produce significant improvements in behavioral outcomes, including episodic memory and anxiety-like behavior. Only a modest reduction in susceptibility to PTZ-induced seizures was observed. The authors proposed that the lack of efficacy may be explained by the intracellular localization of alglucosidase alfa, which is specifically targeted to lysosomes, whereas Lafora bodies accumulate within the cytoplasm and are therefore inaccessible to the enzyme [72].

On the other hand, the same research group investigated the induction of laforin expression by ICV injection of a rAAV vector containing the coding region of the human *EPM2A* gene (rAAV2/9-CAGhEPM2A). This study demonstrated robust neuronal transduction and sustained laforin expression throughout the CNS bypassing the BBB. Remarkably, treated animals exhibited a substantial reduction in LB accumulation, attenuation of epileptiform

activity, improved electrophysiological parameters, and significant correction of behavioral abnormalities. Quantitative proteomic and phosphoproteomic analyses further revealed normalization of multiple molecular pathways associated with neurodegeneration, synaptic dysfunction, and cellular stress, suggesting that restoration of laforin activity exerts pleiotropic therapeutic effects extending beyond glycogen metabolism alone, if compared to the WT both after 3 and 9 months of treatment [73–75]. These findings are particularly significant because they demonstrate, for the first time, that replacement of the missing disease gene can reverse several pathological abnormalities in vivo rather than merely preventing additional glycogen accumulation. Subsequently, the same research group investigated early intravenous (IV) administration of rAAV2/9P31-CAG-hEPM2A (rAAVP31-hEPM2A) and rAAV2/9P31-CAG-hEPM2B (rAAVP31-hEPM2B) vectors, encoding the respective human genes.

In LD mouse models, this approach reduced memory deficits, glial activation and LB formation. Moreover, no hepatotoxic effects or long-term activation of the adaptive immune system were detected, suggesting that this strategy may be encouraging for a safer treatment of this rare disease.

A recent study conducted by Alao et al. investigated the safety of intrathecal AAV9-based gene therapy for malin-deficient LD gene delivered via the cerebrospinal fluid (CSF). Using AAV-mediated gene delivery in *EPM2B* knockout mice models, they evaluated whether increasing the expression of either gene could effectively restore protein function without inducing pathological changes. The study demonstrated that *EPM2A* overexpression significantly reduces LB formation and neuroinflammatory markers in both the brain and muscles (36–45% and~50%, respectively). However, the overexpression of laforin induced the formation of LB, particularly in the dorsal root ganglia, leading to potential toxicity. This toxic effect is more pronounced with high doses and local CSF administration, whereas lower doses are less toxic but also less effective. The study suggests that *EPM2B* represents a promising candidate for gene therapy while *EPM2A*-based approaches require tight regulation of transgene expression or alternative delivery systems to avoid promoting glycogen aggregation and disease pathology [70].

Varea et al. [76] induced malin expression at early and advanced stages (4- and 11-month-old KO mice, respectively) in a malin-deficient mouse (malinKO). Restoration of malin expression did not promote the clearance of pre-existing LBs but prevented the formation of new inclusions in both brain and muscle tissue.

Moreover, malin restoration also triggers the degradation of muscle glycogen synthase and laforin bound to LBs, suggesting that malin interacts with laforin and contributes to the degradation of LB. As known, a fundamental aspect of LD is neuroinflammation. Malin restoration seems to reduce the activation of microglia and inflammatory genes; this might be the result of blocking of the formation of new LBs but also of the changes in the protein composition of the remaining glycogen aggregates. These results show a promising therapeutic strategy for malin-deficient patients such, for the first time, it has proven its effectiveness at advanced stages, and it provides a proof-of-concept for the development of a malin gene therapy-based approach for LD [76].

Hence, the use of high vector doses has been associated with dose-dependent toxicities, including hepatotoxicity, dorsal root ganglion pathology, thrombotic microangiopathy, and immune-mediated adverse events, highlighting the need to further optimize vector design and dosing [77,78]. Another important limitation is pre-existing immunity against AAV capsids, which can reduce transduction efficiency and currently limits vector re-administration [79]. Finally, unlike GYS1-targeting ASOs, which act independently of the underlying genotype, gene replacement requires mutation-specific therapeutic constructs targeting either *EPM2A* or *NHLRC1*, necessitating precise molecular diagnosis before treat-

ment can be implemented [80,81]. Beyond these concerns, achieving sufficient CNS-wide transduction while avoiding excessive transgene expression remains a major challenge. Long-term studies will also be essential to assess the durability of therapeutic effects and identify potential delayed adverse events, particularly given the limited possibility of vector re-administration [77–79].

7. RNA Therapeutics

Two complementary studies investigated GYS1 suppression as a disease-modifying strategy for LD, aiming to reduce abnormal glycogen synthesis rather than correct the underlying genetic defect. Their findings established glycogen synthase as a key therapeutic target, demonstrating that partial GYS1 suppression can substantially ameliorate neuropathological hallmarks and supporting gene-silencing approaches as promising therapies for LD.

In the first study, the authors employed an AAV9-mediated *Staphylococcus aureus* Cas9 (*SaCas9*) genome-editing approach to disrupt *Gys1* expression in adult mouse models of LD and APBD. ICV administration resulted in a significant reduction in Gys1 expression, and a marked decrease in polyglucosan inclusions/LBs, attenuation of astrogliosis and microgliosis, and reduced expression of neuroinflammatory markers. Importantly, these therapeutic effects were achieved when treatment was initiated in adult animals after pathological glycogen deposits had already formed, demonstrating that lowering glycogen synthase activity can modify established disease pathology rather than merely preventing its onset.

Building on this proof-of-concept, the subsequent study developed a more clinically translatable strategy based on an AAV-delivered artificial microRNA (amiRNA) targeting Gys1. Following ICV administration, amiRNA-mediated gene silencing produced a robust reduction in *Gys1* mRNA and protein levels and significantly decreased LB accumulation and neuroinflammation in adult LD and APBD mouse models. Unlike the CRISPR/Cas9 approach, this strategy avoids permanent genome editing while achieving comparable therapeutic efficacy, thereby providing a potentially safer platform for long-term clinical application [60,82].

8. Antibody-Enzyme Fusion Strategies: Intracellular Degradation of Pathogenic Polyglucosans

Antibody-enzyme fusions (AEFs) represent a distinct therapeutic strategy for polyglucosan disorders, aiming to degrade preexisting intracellular inclusions rather than simply reducing their formation [83]. Early foundational studies conducted by Brewer et al. and Zhou et al. introduced the concept of using cell penetrating antibodies to deliver enzymatic cargo directly into the cytoplasm, overcoming the limitations of conventional enzyme replacement.

In LD, the prototype AEF VAL-0417 couples a penetrating antibody with α amylase to digest LBs. Initial preclinical work demonstrated reduced polyglucosan burden and partial metabolic normalization, providing proof of concept for intracellular substrate degradation [84,85].

AEFs are conceptually complementary to substrate reduction approaches such as GYS1 targeting ASOs, which prevent new glycogen accumulation but do not clear existing inclusions. Combined strategies may therefore be particularly relevant in symptomatic patients [86].

The modular nature of AEFs suggests potential applicability to other disorders characterized by abnormal glycogen or polyglucosan, including APBD and GSD IV. Key challenges remain, CNS delivery, immunogenicity, substrate specificity, and intracellular trafficking,

but the platform exemplifies a broader therapeutic paradigm focused on direct removal of pathogenic substrates within affected cells [84,85].

The first clinical evaluation of VAL-1221, a glycogen-degrading antibody–enzyme fusion designed to promote intracellular polyglucosan degradation, was conducted in five patients with intermediate-to-advanced LD through a 12-month compassionate-use program. Patients received IV infusions of VAL-1221 (20 mg/kg every other week), and treatment was assessed by comprehensive clinical, electrophysiological, neuroimaging, pharmacokinetic, and metabolomic analyses. Although the therapy was generally well tolerated, with only mild infusion-related adverse events, no meaningful clinical benefit was observed, and all patients continued to show progressive neurological deterioration. Pharmacokinetic analyses demonstrated that VAL-1221 was undetectable in the CSF, while metabolomic profiling revealed no significant changes following treatment, indicating that IV administration failed to achieve adequate central nervous system exposure. These findings provide important evidence that, despite the favorable safety profile of VAL-1221, systemic delivery is insufficient to modify disease progression. Consequently, the study redirects the development of antibody-enzyme fusion therapies toward direct CNS delivery strategies capable of overcoming the BBB and achieving therapeutically relevant concentrations within the brain [87].

Table 1. Emerging disease-modifying therapeutic strategies for Lafora disease and related polyglucosan disorders (2022–2026).

Treatment	Mechanism/Target	Evidence Stage/Model	Main Findings	Reference
Metformin	AMPK activation; modulation of autophagy, inflammation and oxidative stress	LD mice; 18 LD patients (observational data)	Improved neurological and pathological outcomes in mice; preliminary association with slower clinical progression	Burgos et al., 2023 [29]
Trehalose	Autophagy enhancement; proteostasis modulation	LD zebrafish (epm2a knockout)	Improved locomotion; reduced apoptosis, glycogen burden and inflammation	Della Vecchia et al., 2022 [40]
Dapagliflozin	SGLT2 inhibition; metabolic and autophagic–lysosomal modulation	LD zebrafish (*Epm2a* knockout)	Improved behavior and seizure-like activity; reduced oxidative and inflammatory markers; LB effects untested	Della Vecchia et al., 2025 [42]
Empagliflozin	SGLT2 inhibition	LD zebrafish; additional mechanistic study protocol	Improved locomotor and excitability phenotypes in zebrafish; further mechanism/efficacy work pending	Della Vecchia et al., 2025 [42]; d'Orsi et al., 2025 [43]
Canagliflozin	SGLT2 inhibition	LD zebrafish (*Epm2a* knockout)	Improved locomotor/excitability phenotypes; no evidence yet for LB reduction or clinical efficacy	Della Vecchia et al., 2025 [42]
Fingolimod	S1PR1 modulation; immunomodulation	LD mice (*Epm2b*−/−)	Reduced neuroinflammation and T-cell infiltration; improved behavioral outcomes	Rubio et al., 2024 [44]
Dimethyl fumarate	Nrf2/HCAR2-linked immunomodulation	LD mice (*Epm2b*−/−)	Attenuated astrogliosis, but limited effects on inflammatory and behavioral outcomes	Rubio et al., 2024 [44]

Table 1. *Cont.*

Treatment	Mechanism/Target	Evidence Stage/Model	Main Findings	Reference
Compound 9c (SPAA)	Selective competitive inhibition of laforin phosphatase	Biochemical/cellular assays; mouse pharmacokinetics	Potent chemical probe of laforin activity; no disease-modifying efficacy demonstrated	Lin et al., 2025 [48]
GHF-201 (144DG11)	LAMP1 interaction; enhanced autophagic glycogen catabolism	APBD cells/mice; 3 APBD patients (compassionate use); LD mice	APBD: preclinical benefits and preliminary human biomarker findings; LD mice: no reduction in polyglucosans or gliosis	Kakhlon et al., 2021, 2026 [34,49]; Wu et al., 2024 [8]
GYS1-targeting ASO	RNase H1-mediated *Gys1* mRNA reduction	LD mouse models	Reduced LB formation/progression and epileptiform discharges; no human efficacy data	Ahonen et al., 2021 [81]; Donohue et al., 2023 [55]
GBE1 splice-switching ASOs	Correction of aberrant GBE1 splicing	APBD patient-derived fibroblasts	Restored correct splicing, GBE1 protein levels and enzymatic activity in vitro	Thomas et al., 2025 [57]
EPM2A AAV gene replacement	Restoration of laforin expression	LD mouse models	Reduced LB and neurological phenotypes in several studies; excess laforin expression caused LB formation/DRG safety concerns	Zafra-Puerta et al., 2024, 2025 [72–75]; Alao et al., 2026 [70]
EPM2B AAV gene replacement	Restoration of malin expression	LD mouse models	Prevented additional LB accumulation and improved selected outcomes; preclinical safety findings favorable in tested settings	Varea et al., 2022 [76]; Zafra-Puerta et al., 2025 [73,74]; Alao et al., 2026 [70]
AAV-delivered Gys1 amiRNA	RNA-mediated suppression of glycogen synthesis	LD and APBD mouse models	Reduced glycogen accumulation, polyglucosan bodies and neuroinflammatory markers	Gumusgoz et al., 2022 [82]
VAL-1221	Antibody–enzyme fusion for intracellular glycogen degradation	5 LD patients (compassionate use)	Generally tolerated but no meaningful clinical benefit; insufficient CNS exposure after IV dosing	Muccioli et al., 2025 [87]
Alglucosidase alfa	Lysosomal glycogen-degrading enzyme	LD mouse models (*Epm2a*−/− and knock-in)	No significant LB reduction or behavioral improvement; modest effect on seizure susceptibility	Zafra-Puerta et al., 2024 [72,75]

9. Conclusions

Recent advances in the understanding of glycogen metabolism and LD pathogenesis have substantially reshaped its therapeutic landscape, moving the field from predominantly symptomatic management toward mechanism-based interventions. Pathological glycogen accumulation has emerged as a central therapeutic target, with strategies aimed at reducing its synthesis, restoring the defective laforin–malin pathway, or promoting the degradation of accumulated polyglucosans. In particular, partial suppression of GYS1 has shown consistent efficacy in experimental models, while recent progress in ASO- and RNA-based approaches, gene replacement, and intracellular substrate-degradation technologies has considerably broadened the range of potential disease-modifying interventions.

Despite these promising advances, most therapeutic strategies discussed in this review remain at the preclinical stage, with efficacy demonstrated primarily in cellular and animal models. Human evidence is currently limited to preliminary observations involving metformin in LD and **GHF-201** in a small number of patients with APBD. Therefore, although these approaches provide valuable proof-of-concept, their clinical efficacy, safety, and long-term benefits remain to be established through adequately designed clinical studies.

The timing and mechanism of therapeutic intervention are also likely to be critical. Preventing or limiting pathological glycogen accumulation may be particularly effective at early disease stages, whereas established LBs appear considerably more difficult to remove. Beyond therapeutic development, additional challenges inherent to ultra-rare diseases include timely diagnosis and the limited availability of epidemiological and natural-history data, which may be partly addressed through systematic data collection in disease registries. From a clinical perspective, metformin currently represents one of the few approaches investigated in patients with LD, although its efficacy remains to be confirmed in controlled studies. Other emerging strategies, particularly GYS1-targeting ASOs and gene-based therapies, remain at the preclinical stage and require further investigation to address challenges related to CNS delivery, safety, and long-term efficacy.

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Abbreviations

The following abbreviations are used in this manuscript:

GSDs	Glycogen storage diseases
CNS	Central nervous system
LD	Lafora disease
APBD	Adult Polyglucosan Body Disease
GSD IV	Glycogen Storage Disease Type IV
ASOs	Antisense oligonucleotides
IC_{50}	50% inhibitory concentrations
PTG	Protein targeting to glycogen
TNF	Tumor Necrosis Factor
IL-6	Interleukin-6
JAK2	Janus Kinase 2
LBs	Lafora Bodies
AMPK	AMP-activated protein kinase
PTZ	pentylenetetrazole
EMA	European Medicines Agency

FDA	Food and Drug Administration
LESS	Lafora Epilepsy Severity Scale
SGLT2	Sodium–glucose cotransporter-2
FGD	Fingolimod
DMF	Dimethyl fumarate
LAMP1	Lysosomal-Associated Membrane Protein 1
BBB	Blood–brain barrier
SPAA	Sulfophenyl acetic amide
PTP	Protein tyrosine phosphatases
mRNA	messenger RNA
pre-mRNA	precursor mRNA
ICV	intracerebroventricular
WT	wild-type
AAV	Adeno-associated virus
IV	Intravenous
CSF	Cerebrospinal fluid
amiRNA	artificial microRNA
AEFs	Antibody-enzyme fusions

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