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## Lafora disease — from pathogenesis to treatment strategies

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### Abstract

Lafora disease is a severe, autosomal recessive, progressive myoclonus epilepsy. The disease usually manifests in previously healthy adolescents, and death commonly occurs within 10 years of symptom onset. Lafora disease is caused by loss-of-function mutations in *EPM2A* or *NHLRC1*, which encode laforin and malin, respectively. The absence of either protein results in poorly branched, hyperphosphorylated glycogen, which precipitates, aggregates and accumulates into Lafora bodies. Evidence from Lafora disease genetic mouse models indicates that these intracellular inclusions are a principal driver of neurodegeneration and neurological disease. The integration of current knowledge on the function of laforin-malin as an interacting complex suggests that laforin recruits malin to parts of glycogen molecules where overly long glucose chains are forming, so as to counteract further chain extension. In the absence of either laforin or malin function, long glucose chains in specific glycogen molecules extrude water, form double helices and drive precipitation of those molecules, which over time accumulate into Lafora bodies. In this article, we review the genetic, clinical, pathological and molecular aspects of Lafora disease. We also discuss traditional antiseizure treatments for this condition, as well as exciting therapeutic advances based on the downregulation of brain glycogen synthesis and disease gene replacement.

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Lafora disease (Online Mendelian Inheritance in Man (OMIM) #254780) is a rare autosomal recessive and severe form of progressive myoclonus epilepsy. After onset, which usually occurs during late childhood or early adolescence, Lafora disease is invariably fatal, typically within 10 years<sup>1,2</sup>. The condition was first described by Lafora and Glück over 100 years ago<sup>3</sup>. A post-mortem study showed profuse accumulation of small inclusion bodies in many tissues, including the brain. These inclusions, subsequently termed Lafora bodies, became the hallmark of the disease. They were shown to be composed primarily of

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#### Author contributions

F.N., S.J.A., S.N. and S.M. researched data for the article. F.N., S.N. and B.A.M. made substantial contributions to discussions of the content. All authors wrote the article and reviewed and/or edited the manuscript before submission.

#### Competing interests

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abnormal glycogen<sup>4</sup>, placing Lafora disease in the context of glycogen metabolism disorders.

The affected genes in Lafora disease — *EPM2A*, which encodes laforin glucan phosphatase (henceforth termed laforin) and *NHLRC1* (also known as *EPM2B*), which encodes E3 ubiquitin protein ligase 1 (henceforth termed malin) — were discovered only two decades ago<sup>5–7</sup> (FIG. 1). The exact roles of laforin and malin in glycogen metabolism are still under investigation. However, recent progress affords an improved understanding of the disease mechanisms, leading to the identification of new therapeutic avenues for what is arguably the severest of the epilepsies.

This Review provides a broad overview of Lafora disease, including its clinical features, genetics and existing management strategies. Our current understanding of Lafora disease pathogenesis is outlined, laying the groundwork for discussion of new disease-mechanism-based therapeutic strategies.

## Clinical features of Lafora disease

At the time of onset, Lafora disease is difficult to distinguish from idiopathic generalized epilepsies<sup>8</sup>. Apparently healthy older children or teenagers start having seizures, which can initially be controlled with antiepileptic drugs (AEDs)<sup>8,9</sup>. Several types of seizure typically occur in patients with Lafora disease, including myoclonic, occipital, generalized tonic-clonic, absence and atonic seizures<sup>10,11</sup>. In retrospect, parents often recall that their child had experienced isolated febrile or non-febrile seizures earlier in childhood<sup>10</sup>. Additional symptoms in the first few years comprise behavioural changes, confusion, depression, dysarthria, ataxia and intellectual decline (BOX 1). Over time, the seizures become more frequent and increasingly intractable. Behavioural and cognitive deterioration increases, gradually leading to dementia. Eventually, the patient enters a vegetative state with continuous myoclonus and requires tube feeding and artificial respiration. Death commonly results from status epilepticus or aspiration pneumonia and other complications of chronic neurodegeneration<sup>2,11</sup>.

## Genetics

Many types of pathogenic variant have been identified in the genes that encode laforin and malin including missense, nonsense and frameshift mutations, as well as some larger deletions, particularly in *EPM2A*<sup>12</sup>. Human laforin consists of two functional domains: an amino-terminal family 20 carbohydrate-binding module (CBM20) and a carboxy-terminal dual-specificity phosphatase (DSP) domain. Malin is an E3 ubiquitin ligase containing a RING domain and six NHL repeats, both of which are typical for this family of enzymes (FIG. 1). In general, mutations in *EPM2A* and *NHLRC1* are distributed evenly across both genes, with no particular clustering in the functional domains mentioned above (see The Lafora Progressive Myoclonus Epilepsy Mutation and Polymorphism Database<sup>13</sup>).

Studies have shown that certain mutations within the DSP domain of laforin compromise its phosphatase activity, which might render the protein pathogenic<sup>14,15</sup>. However, two independent studies demonstrated that phosphatase activity is not required to rescue laforin-

deficient mice from Lafora disease<sup>16,17</sup>. Evidently, in many cases, other properties of laforin, including glycogen binding, subcellular localization and the interaction with malin or protein phosphatase 1 regulatory subunit 3 C (PP1 subunit R5; also known as PTG), are additionally affected by DSP mutations<sup>14,15,18,19</sup>. For example, mutations in the DSP, such as Gly279Ser, Gln293Leu, Tyr294Asn and Pro301Leu<sup>14</sup>, can lead to decreased glycogen binding, although the CBM20 domain is not directly affected. Conversely, mutations within the CBM20, such as Trp32Gly, Phe84Leu and Arg108Cys<sup>14</sup>, can lead to loss of phosphatase activity. Together, these findings clearly show that the overall effect of a mutation on laforin function cannot be deduced solely from the mutation site. Furthermore, the role of laforin in glycogen metabolism and Lafora disease is not confined to its function as a phosphatase.

In a few patients with Lafora disease, sequence analysis of *EPM2A* and *NHLRC1* coding regions reveals no mutation in either gene<sup>20–23</sup>. In such cases, the disease could be caused by mutations in non-coding regulatory regions, such as promoter or intronic regions<sup>20,24</sup>. Mutations in non-coding regions might also explain some cases in which only one heterozygous mutation in the coding region could be identified<sup>23</sup>. Patients who carried an apparently homozygous mutation in *NHLRC1* but had one parent who lacked mutations in this gene were found to have large deletions in one allele, which were undetectable by PCR<sup>25</sup>. In addition, disease-causing mutations in a third locus have been proposed in cases in which causative involvement of *EPM2A* or *NHLRC1* was excluded<sup>24</sup>. Further analysis revealed a mutation in the PR domain zinc-finger protein 8 gene (*PRDM8*) in a single family<sup>26</sup>. Of note, patients with *PRDM8* mutations presented with an atypical form of Lafora disease, characterized by early childhood onset and negative skin (but positive muscle) biopsies<sup>26</sup>. Finally, some patients with no *EPM2A* or *NHLRC1* mutations might not actually have Lafora disease but were misdiagnosed on the basis of a false-positive skin biopsy (see ‘Diagnostic strategy’ section below). Therefore, it seems likely that essentially all classic cases of Lafora disease can be explained by mutations in *EPM2A* or *NHLRC1*.

The clinical features of classic Lafora disease are similar in most patients<sup>10,12</sup>, although the time of onset and the rate of disease progression can vary substantially<sup>25,27</sup>. In the past, attempts have been made to establish genotype-phenotype correlations<sup>12,18</sup>. One example is the early-onset learning disorder phenotype in some patients with Lafora disease who harbour a mutation in exon 1 of *EPM2A*<sup>18,28</sup>, although not all patients with such mutations have this atypical subsyndrome<sup>21,29</sup>. Another example is the observation that patients with *NHLRC1* mutations — in particular, the Asp146Asn mutation — tend to live longer than those with *EPM2A* mutations<sup>20,21,23,30,31</sup>. However, some patients with *NHLRC1* mutations have very severe phenotypes<sup>29,32–34</sup>.

What factors make genotype-phenotype correlation studies so difficult? The fact that Lafora disease is rare and shows considerable mutational heterogeneity, with more than 90 known pathogenic variants in *EPM2A* and almost 80 in *NHLRC1* (REF<sup>13</sup>), hampers progress in understanding the genetic epidemiology of the condition. Various combinations of compound heterozygosity for mutations<sup>12,20,29,35</sup> further complicate correlation studies. In addition, the quality of available care has a strong impact on the frequency of disease complications and survival and varies between countries and health systems<sup>10,12,18</sup>. Moreover, evidence indicates that genetic factors other than the disease-causing *EPM2A* or

*NHLRC1* mutations, such as modifier genes, can substantially modulate the course of the disease<sup>36</sup>. For example, of two siblings with homozygous exon 1 mutations in *EPM2A*, only one presented with the early-onset learning disorder described above, even though both siblings carried the same pathogenic variant<sup>35</sup>. Another example is the highly variable age of onset among siblings carrying the same mutation<sup>25,27</sup>. Candidate factors that influence the pathogenesis of Lafora disease include interacting partners of laforin and/or malin. Indeed, a variant of PP1 subunit R5 — a protein that usually interacts with the laforin-malin complex — was suggested to be responsible for a milder course of Lafora disease<sup>37</sup>. This protein variant exhibits reduced function, and its effects in humans are consistent with the observation that knockout of the gene encoding PP1 subunit R5 leads to rescue of the Lafora disease phenotype in mice<sup>38</sup>.

## Pathogenesis

Understanding the pathogenesis of Lafora disease depends on providing a mechanistic link between a deficiency in laforin or malin and the neurodegenerative disease. Studies of the pathological hallmark of the disease, the Lafora bodies, provided insight into the affected metabolic pathways even before the genetic causes were found; Lafora bodies were classified as polyglucosan bodies, as they are largely composed of glucan chains and, hence, are chemically similar to glycogen<sup>4</sup> (FIG. 2a). Intriguingly, in contrast to cytosolic glycogen, these polyglucosans are water-insoluble. The exact roles of laforin and malin are still under investigation, but they are likely to be involved in processes that prevent the accumulation of insoluble glycogen-like particles (FIG. 2b). The sections that follow discuss Lafora body accumulation as the cause of Lafora disease, review the proposed roles of laforin and malin in glycogen metabolism (BOX 2) and propose a model of Lafora disease pathogenesis whereby laforin and malin act as part of a glycogen quality control mechanism to prevent glycogen insolubility.

## Lafora bodies

The accumulation of dense cytoplasmic aggregates is a feature of many neurodegenerative diseases, including Parkinson disease and Alzheimer disease<sup>39,40</sup>, and the accumulation of Lafora bodies seems to be the primary cause of Lafora disease progression. This conclusion has been supported by several studies in mouse models of Lafora disease, which lack either laforin or malin and recapitulate the human disease<sup>41,42</sup>. Genetic approaches that reduce or abolish glycogen synthesis prevent Lafora body formation and rescue other features of the Lafora disease phenotype in mice, including autophagy impairment, neurodegeneration and seizure susceptibility<sup>38,43,44</sup>. In addition, overexpression or increased activation of the glycogen-synthesizing enzyme glycogen synthase in the presence of laforin and malin can result in polyglucosan body formation, as well as down-stream effects such as impaired autophagy and neuro- degeneration<sup>43,45</sup>. Evidence of a link between impaired autophagy and neurodegeneration<sup>46–48</sup> completes the current view on the pathogenesis of Lafora disease. The roles of laforin and malin in glycogen metabolism need to be unravelled to enable us to understand the mechanisms that lead to Lafora body formation and accumulation.

## The role of laforin as a phosphatase

CBM20s are found in various glucan chain-binding proteins across many species<sup>49,50</sup>, and the presence of this module in laforin places this protein in the context of glycogen metabolism (BOX 2; FIGS. 1,2c). Laforin is known to bind to glycogen<sup>51</sup>, but how it prevents glycogen insolubility and protects against Lafora disease is open to question.

The identification of a DSP in laforin and the fact that protein phosphorylation has a substantial role in the regulation of the chain-elongating enzyme glycogen synthase implicated laforin in the regulation of glycogen synthesis. Glycogen synthase kinase 3 (GSK3) is activated by dephosphorylation and itself phosphorylates and inactivates glycogen synthase<sup>52</sup>. Yeast two- hybrid and cell culture overexpression experiments showed that laforin interacts with and dephosphorylates the muscle isoform of GSK3 (REF<sup>53</sup>). A defect in laforin, resulting in insufficient GSK3 activation, might therefore lead to abnormally high glycogen synthase activity. However, no increase in glycogen synthase activity could be demonstrated in a laforin-deficient mouse model of Lafora disease<sup>42</sup>.

Another interesting discovery was the ability of laforin to dephosphorylate glycogen (FIG. 2c). Over 30 years ago, glycogen was discovered to contain small amounts of covalently bound phosphate<sup>54</sup>. The role of glycogen phosphate gained importance when laforin was shown to act as a glucan phosphatase<sup>55,56</sup>. Glycogen in Lafora disease mice was found to have elevated levels of covalently bound phosphate and to contain an abnormally high proportion of long glucan chains<sup>57,58</sup>. On the basis of these observations, it was hypothesized that an excess of glycogen phosphate lies at the root of Lafora disease pathogenesis, and that laforin keeps glycogen phosphate levels low to prevent abnormal glycogen structure, glycogen insolubility and, hence, Lafora disease<sup>59</sup>. However, more recent studies contradict this hypothesis by showing that the laforin-deficient Lafora disease mouse model can be rescued by overexpression of laforin with a mutated DSP lacking phosphatase activity. In fact, the glycogen in the rescued mice was still hyperphosphorylated, as in the laforin knockout, but the chain length was normal, Lafora bodies were absent, and the behavioural phenotype was normalized<sup>16,17</sup>. These findings largely excluded glycogen hyperphosphorylation as the underlying cause of Lafora disease.

## The laforin-malin interaction

Investigations into the role of laforin in cell metabolism revealed several interaction partners for this protein, some of which were related to endoplasmic reticulum stress<sup>60</sup>, protein clearance<sup>61,62</sup>, iron homeostasis<sup>63</sup> or tumour suppression<sup>64</sup>. Besides GSK3, additional laforin interaction partners with a close connection to glycogen metabolism were identified, including glycogen synthase<sup>55</sup>, subunits R4, R5 and R6 of the glycogen synthase activator PP1 (REFS<sup>14,55,65</sup>) and AMP-activated kinase (AMPK), a protein that is involved in glycogen phosphorylase regulation. The array of putative interaction partners implies a complex role for laforin, involving multiple functions<sup>66</sup>.

The interaction of laforin with malin is of particular interest and has been supported by a large number of studies<sup>14,53,65,67,68</sup>. The existence of a patient mutation that causes Lafora disease merely through the loss of the laforin-malin interaction<sup>69</sup> emphasizes the relevance

of this interaction in vivo. Moreover, the similar clinical progression in patients with *EPM2A* and *NHLRC1* mutations is consistent with a crucial role for both proteins in a single functional complex. The laforin-malin complex was demonstrated to incorporate lysine 63 (K63)-linked polyubiquitin chains<sup>19,70,71</sup>, which principally promote autophagic inclusion and degradation of ubiquitylated targets<sup>72–74</sup>. In agreement with evidence for proteasomal degradation of laforin-malin targets in the cytosol<sup>65,69,75</sup>, this finding implies that targets of the laforin-malin complex are generally subjected to degradation. Interestingly, the targets of malin-mediated ubiquitylation that have been demonstrated ex vivo include glycogen synthase<sup>69</sup> and PP1 subunit R5 (REF<sup>65</sup>). Glycogen synthase drives glycogen chain elongation, and PP1 subunit R5 indirectly activates glycogen synthase by targeting PP1 to glycogen<sup>76</sup>. Therefore, both proteins help to determine a pivotal aspect of glycogen structure — the balance between chain elongation and branching (Fig. 2c).

### The importance of glycogen structure

Elongation and branching of glucan chains, as well as chain degradation and debranching, are enzymatic features that are not restricted to glycogen metabolism but are also involved in starch metabolism in plants<sup>77</sup>. Glycogen and amylopectin, the main component of plant starch, are both polyglucans<sup>78,79</sup> (Fig. 2a). Unlike plant starch, however, glycogen is normally water-soluble. Glycogen and amylopectin both consist of glucan chains with a wide distribution of chain lengths<sup>17,80</sup>, but the average chain length is generally higher in amylopectin than in glycogen<sup>81</sup>. In glycogen, branching points are essentially distributed evenly, whereas in amylopectin, they are arranged in clusters<sup>82,83</sup>, giving rise to regions with fewer branching points where neighbouring glucan chains form double helices. In turn, these structures form semi-crystalline layers that render the entire starch granule water-insoluble<sup>81,83,84</sup>. Interestingly, genetically engineered plants with impaired clustering of branching points produce a soluble form of polyglucan, termed phytoglycogen<sup>85,86</sup>. Thus, the frequency of branching points, which is controlled by the concerted action of glycogen synthase and glycogen branching enzyme (GBE) in the case of glycogen metabolism, seems to determine the water solubility of a polyglucan<sup>87</sup> (Fig. 2c).

Glycogen synthase is regulated post-translationally through complex mechanisms involving phosphorylation and allosteric effectors<sup>88</sup>, which allow a fine-tuned balance of chain elongation and branching. In mice with laforin or malin deficiency, glycogen shows structural abnormalities consisting of substantially increased proportions of long glucan chains<sup>58</sup>, suggesting a role for the laforin-malin complex in the regulation of glycogen chain elongation.

### Glycogen quality control

The laforin-malin complex is thought to downregulate glycogen chain elongation, probably by targeting both glycogen synthase and PP1 subunit R5, which is involved in glycogen synthase activation, to degradation<sup>65,69</sup>. Accordingly, in Lafora disease, insufficient downregulation of this process would lead to an imbalance of elongation and branching reactions, resulting in overly long glucan chains, double-helix formation and, hence, glycogen insolubility and deposition as Lafora bodies. However, studies showing that levels of glycogen synthase and PP1 subunit R5 were not substantially altered in tissue lysates



from Lafora disease mice cast doubt on whether the two proteins are substrates of the laforin-malin complex in vivo<sup>42</sup>. This apparent inconsistency might be resolved by assuming a locally focused action of the laforin-malin complex, targeting glycogen synthase and PP1 subunit R5 only on a small subgroup of glycogen molecules that are especially prone to precipitation<sup>87</sup>. Such subcellular changes would not be detected if protein levels and activity are quantified in whole-tissue lysates<sup>42</sup>.

This theory is corroborated by the fact that normal glycogen is a heterogeneous mixture of macromolecules. The range of molecule sizes within one preparation spans at least one order of magnitude, and evidence exists that some molecules are composed of longer chains than others<sup>58,89</sup>. As long chains promote polyglucan insolubility, glycogen molecules might vary in their propensity to undergo precipitation (FiG. 3a,b). The differences between glycogen molecules are likely to be inherent to normal glycogen metabolism and could be caused by subcellular inhomogeneity of constituents that affect local glycogen synthase activity, including the enzyme itself, the allosteric activator glucose 6-phosphate and the substrate UDP-glucose. Subcellular inhomogeneity has been demonstrated for a number of metabolites, including ATP, reduced NADH and glucose<sup>90-92</sup>.

Through its CBM20, laforin preferentially binds polyglucans composed of longer glucan chains. This fact is illustrated by the predilection of laforin for solubilized potato starch over glycogen with respect to substrate binding<sup>51</sup> and is consistent with the strong enrichment of laforin in Lafora bodies in malin-deficient Lafora disease mice<sup>93</sup>. Via its interaction with laforin, malin is likely to be sequestered preferentially to glycogen molecules with a larger proportion of long chains (FiG. 3a), where it ubiquitylates glycogen synthase and PP1 subunit R5 for subsequent degradation. Accordingly, chain elongation would be specifically decreased at glycogen molecules with an increased probability of precipitation, leading to a localized relative increase in branching frequency. This phenomenon would result in shorter chains, higher solubility and, through avoidance of precipitation, availability of these glycogen molecules for normal glycogen degradation<sup>87</sup> (FiG. 3c). In addition to this putative role of the laforin-malin complex in avoiding glycogen insolubility, laforin removes glycogen phosphate to allow complete glycogen degradation by glycogen phosphorylase and glycogen debranching enzyme<sup>80</sup>.

In addition to the strategy of preventing the formation of glycogen with a high risk of precipitation, the laforin-malin complex could be involved in an autophagic process to remove abnormal or already insoluble glycogen. Starch-binding domain-containing protein 1 (STBD1) anchors glycogen to intracellular membranes and also interacts with autophagic proteins<sup>94</sup>. STBD1 could have a role in targeting malstructured glycogen to lysosomal degradation, although its function does not seem to be impaired in Lafora disease. Cell culture experiments have shown that the laforin-malin complex interacts with p62 (also known as sequestosome 1), an autophagy-related adaptor protein that binds ubiquitylated proteins that are targeted to autophagy<sup>68</sup>. Lafora body-containing tissue does not always exhibit a general defect in autophagy, and if present, this defect seems to be secondary to glycogen accumulation<sup>17,43,45,80,95</sup>. However, it is conceivable that in mammalian cells, several layers of glycogen quality control are established to avoid deposition and accumulation of abnormal glycogen. The first layer is avoidance of glycogen precipitation

through tight local regulation of glycogen synthase, with an evident role for the laforin-malin complex. A putative second layer would be the removal of abnormal and precipitated glycogen, also possibly involving the laforin-malin complex. In the absence of a functional laforin-malin complex, cellular glycogen quality control is impaired, with fatal consequences, as observed in Lafora disease.

## Diagnostic strategy

Several factors, including the rare nature of the disease, cultural consanguinity, founder effects and limited treatment options, pose considerable challenges for clinicians and caregivers when working with patients with Lafora disease and their families. Diagnosis is usually based on clinical findings (described above), EEG abnormalities<sup>2,8,96</sup> with normal brain MRI<sup>97</sup>, and diastase-resistant periodic acid-Schiff (PAS-D)-positive skin biopsies, revealing the presence of Lafora bodies<sup>29,98</sup> (Fig. 4). As virtually all cases of Lafora disease can be attributed to pathogenic variants of either *EPM2A* or *NHLRC1* (REFS<sup>20,22,23,99</sup>), targeted genetic testing is the gold standard to confirm the diagnosis. Genetic testing is not only less invasive than skin biopsy but is also more readily available and less expensive. Furthermore, skin biopsy testing is fraught with false positivity. The sweat glands where Lafora bodies form contain equally PAS-D-positive and visually impressive normal secretory contents that are frequently falsely identified as Lafora bodies owing to a lack of experience with the disease in most pathology laboratories<sup>98</sup>. False negativity of skin biopsy in patients with genetically confirmed Lafora disease has also been reported<sup>29</sup>. Therefore, clinicians should await genetic confirmation before disclosing the diagnosis to caregivers and — if appropriate and with the approval of the family — to the young patients.

A diagnosis of Lafora disease can cause severe psychological trauma to patients and their families, including but not limited to feelings of guilt and resentment, fear of younger siblings also becoming affected, and financial costs and concern regarding the availability of treatment options. Consequently, the diagnosis should be followed up with sustained, attentive genetic and psychological counselling and support.

## Current management strategies

Currently, AEDs are the only available treatments that control the severity and frequency of seizures and myoclonus to some degree in patients with Lafora disease. Among these drugs, valproic acid is the mainstay. Other effective medications include topiramate, ethosuximide, phenobarbital, zonisamide, felbamate and benzodiazepines. Most recently, perampanel, a new  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor antagonist AED, was shown to be effective in two single-case studies and a group of ten patients<sup>100–102</sup>.

Besides AEDs, vagal nerve stimulation resulted in temporary cessation of generalized tonic-clonic seizures and status epilepticus in two single-case studies<sup>103,104</sup>. The ketogenic diet was also tried in a group of patients with relatively advanced disease but was shown to be ineffective<sup>99</sup>. This finding was surprising given that the diet converts brain energy usage from glucose to fatty acids, thus presumably reducing the neuronal glucose availability for



glycogen (and Lafora body) synthesis. Unpublished work from our laboratory did show the effectiveness of this diet in a Lafora disease mouse model, and the possibility remains that the failure in the clinical setting was attributable to the overly advanced disease in the treated patients rather than to actual ineffectiveness.

In 2016, the European Commission granted orphan designation and permission to use metformin for the treatment of Lafora disease (European Medicines Agency orphan decision number EU/3/16/1803). Metformin is an activator of AMPK and is widely used to treat type 2 diabetes<sup>105</sup>. AMPK activation is associated with the inhibition of several ATP-consuming pathways, including glycogen synthesis<sup>106,107</sup>. In mice and rats, metformin was shown to have positive effects on neuronal survival and seizure termination<sup>108–110</sup>. Studies in a mouse model of Lafora disease showed that metformin ameliorated neuropathological symptoms, reduced seizure susceptibility and slightly reduced the numbers of Lafora bodies<sup>111,112</sup>. No clinical data are yet available regarding the efficacy of metformin as a treatment for Lafora disease.

The dietary supplement sodium selenate has been shown to reduce neurodegeneration, gliosis, seizure susceptibility and memory loss in a mouse model of Lafora disease<sup>113</sup>. However, a gradual decline in overall motor conditioning following an initial improvement in the treated mice raised doubts about the efficacy of the drug as a potential treatment for Lafora disease.

Aminoglycoside antibiotics, such as gentamicin, can suppress translation termination at premature termination codons<sup>114,115</sup> and could be repositioned for potential use in patients with Lafora disease who have nonsense mutations. However, clinical studies involving patients with cystic fibrosis, Duchenne muscular dystrophy or McArdle disease resulted in various outcomes<sup>115</sup>. At best, only subpopulations of patients benefited from the treatment<sup>116–119</sup>, and the use of aminoglycosides is also limited by adverse effects<sup>120,121</sup>. No preclinical data are available for the use of gentamicin to treat Lafora disease.

## The road to new therapies

### Virus-mediated gene replacement

As discussed above, Lafora disease is primarily a neurodegenerative disease, and the symptoms are caused by Lafora body accumulation in the brain due to deficiency in either laforin or malin<sup>87</sup>. Gene therapy to deliver a functional copy of the defective gene would thus be an obvious option for Lafora disease treatment (Fig. 5). Undoubtedly, gene therapy has considerably advanced the development of treatments for hereditary diseases. Several strategies to introduce a transgene have been developed, including the use of viral vectors, such as adeno-associated virus (AAV)<sup>122</sup> and lentivirus<sup>123</sup>. Other delivery methods, using nanoparticles, liposomes and exosomes, have also been established<sup>124,125</sup>.

AAV has become the vector of choice for gene replacement, owing to its non-pathogenic nature, fairly widespread transduction, long-lasting and high transgene expression, and very low frequency of integration into the host genome<sup>122,123</sup>. Although packing capacity is a limiting factor for AAV-mediated gene delivery<sup>122</sup>, cDNA sequences of *EPM2A* and

*NHLRC1* are below the size limit and can, therefore, be delivered using this vector. The greatest hurdle to AAV-mediated delivery of these genes is the transduction efficiency. As Lafora bodies form throughout the brain, widespread CNS transduction is paramount. The transduction efficiency has been improved through the use of serotypes with high neuronal transduction efficiency, such as AAV9<sup>123</sup>, or modified serotypes, such as AAV-PHP.eB or AAV-PHPS<sup>126</sup>.

AAV9 is known to pass through the blood-brain barrier (BBB)<sup>127</sup>, which makes intravenous delivery possible. However, vector loss through high off-target transduction (mainly accumulation in the liver) is a concern with systemic delivery<sup>123</sup>. Direct CNS delivery — for example, intrathecal administration — would circumvent this problem and enable relatively high transduction throughout the CNS. Intrathecal delivery is considered fairly safe and is routinely used to deliver various drugs to the CNS<sup>128,129</sup>. Delivery via the cerebrospinal fluid might also circumvent loss of the virus by neutralizing antibodies<sup>130</sup>. As AAV infects humans naturally, the pre-existence of neutralizing antibodies leads to reduced transgene expression when the vector is delivered systemically<sup>131,132</sup>. In addition, despite the low immunogenicity of AAV, efficient therapy can only be accomplished once<sup>133</sup>.

A clinical trial using intrathecal AAV administration is ongoing for Batten disease, a late infantile neuronal ceroid lipofuscinosis, and is likely to establish a proof of concept for this delivery method in AAV therapy for CNS disorders. Several other clinical trials are ongoing in CNS disorders<sup>123</sup>. In Lafora disease, early intervention is likely to be the best option; in metachromatic leukodystrophy<sup>134</sup> and adrenoleukodystrophy<sup>135</sup>, gene therapy has been shown to arrest neurodegeneration if administered before extensive CNS damage has occurred.

### Degradation of Lafora bodies

Another therapeutic option for Lafora disease is the degradation of accumulated Lafora bodies (FIG. 5). This goal could be achieved by the delivery of a polyglucan-degrading enzyme to the brain. For instance,  $\alpha$ -amylase is known to break the  $\alpha$ -1,4 glycosidic linkages in glycogen and starch and thus could mediate Lafora body-degradation. Efficient delivery strategies are essential to effectively introduce the Lafora-body-degrading protein into the cells that harbour these inclusions. Fused to a non-toxic form of diphtheria toxin,  $\alpha$ -amylase can be translocated into cells, is active and can degrade intracellular glycogen<sup>136</sup>. Among the vast variety of  $\alpha$ -amylases that are present in different species, an enzyme that both effectively degrades insoluble polyglucans (such as Lafora bodies) under cytosolic conditions and provokes a minimal immune reaction should be selected.

### Reducing brain glycogen synthesis

One of the most promising therapeutic avenues for Lafora disease is the reduction of brain glycogen synthesis (FIG. 5). Partial or full removal of glycogen [starch] synthase, muscle (GYS1) — the glycogen synthase isoform that is expressed in the brain — in Lafora disease mouse models prevented Lafora body formation and led to rescue of the neurological phenotype. These findings imply that Lafora disease could be prevented by inhibiting glycogen synthesis, and that inhibition by ~50% might be sufficient to halt the progression

of the disease<sup>43,137,138</sup>. Patients with loss- of-function *GYS1* mutations, including those with complete loss of GYS1 (GSDOb, OMIM #611556), experience cardiac problems, epilepsy and exercise intolerance<sup>139–141</sup>. However, no health problems were reported in parents of these individuals in whom GYS1 levels were reduced to 50%<sup>140,141</sup>. *Gysl-null* mice have only 10% survival, with pups dying perinatally owing to cardiac dysfunction. However, the mice that do survive have apparently normal heart function, and heterozygous mice, with ~50% GYS1 levels, have no reported health issues<sup>142</sup>.

Another more indirect way to prevent glycogen synthesis is to decrease glycogen synthase activity by reducing levels of PP1 subunit R5, an indirect activator of this enzyme. Removal of PP1 subunit R5 in Lafora disease mice almost fully prevented Lafora body formation, and rescued neurodegeneration and the subsequent myoclonic epilepsy phenotype<sup>38,44</sup>. However, PP1 subunit R5 is only one of several PP1 subunits that promote glycogen activation<sup>55,65</sup>, and removal of this subunit led to a substantial reduction in but not a complete absence of Lafora bodies. In addition, PP1 subunit R5 promotes inactivation of glycogen phosphorylase, which mediates glycogen degradation<sup>76</sup>; therefore, the absence of this subunit might increase glycogen degradation. Targeting of other PP1 subunits in addition to the R5 subunit could be beneficial.

In principle, glycogenin 1 (encoded by the *GYGI* gene) could also be considered as a therapeutic target for Lafora disease owing to its function as the primer for glycogen synthesis<sup>143</sup>. However, data concerning the *Gygl*- null mouse model and patients with loss-of-function mutations in *GYGI* (GSDXV, OMIM #613507) suggest that glycogenin 1 depletion leads to glycogen accumulation, cardiomyopathy and muscle weakness. Therefore, this protein might not be the best therapeutic target for Lafora disease<sup>144–146</sup>.

**Antisense oligonucleotides.**—Antisense oligonucleotides (ASOs) are emerging as an excellent therapy platform, and though not a new concept<sup>147</sup>, ASO therapies have made substantial progress in recent years<sup>148,149</sup>. ASOs are short synthetic nucleic acids that are chemically modified to increase their stability in biological fluids and potency in binding to the target mRNA<sup>147</sup>. ASOs function in several different ways, one of which entails Watson-Crick base pairing with the target mRNA, leading to RNase H-mediated target degradation<sup>148</sup>.

The use of ASO therapy to target the mRNA encoding GYS1, PP1 subunit R5 and/or other PP1 subunits in the brain is a viable option for the treatment of Lafora disease (FIG. 5). As ASOs do not cross the BBB, direct CNS delivery is required in Lafora disease, as for other neurological diseases<sup>150</sup>. Intrathecally delivered ASOs spread widely throughout the brain and spinal cord in mice, rats and nonhuman primates<sup>150–154</sup>. However, ASOs require repeated administration to maintain therapeutic levels because they gradually degrade over time<sup>155</sup>.

ASO therapy targeting the splicing of the *SMN2* gene is already prescribed to patients with spinal muscular atrophy<sup>156,157</sup>, and ASO drugs have also been developed for non-neurological diseases<sup>158</sup>. Clinical trials are ongoing to develop ASO therapies in

amyotrophic lateral sclerosis (targeting *SOD1* and *C9orf72*), Huntington disease (targeting *HTT*), tauopathies (targeting *MAPT*) and Alzheimer disease (targeting *APP*)<sup>149</sup>.

**RNA interference.**—RNA interference (RNAi) is another potential option for post-transcriptional suppression of Lafora disease-related therapeutic targets (FIG. 5). RNAi is achieved by the delivery of artificial short RNAs, such as microRNAs (miRNAs) and short hairpin RNAs (shRNAs), which are designed to pair with their target mRNA, leading to target degradation<sup>159</sup>. Artificial miRNAs and shRNAs are recognized by the short-RNA-processing machinery of the cell and are processed as endogenous small RNAs<sup>160</sup>. RNAi cell toxicity is a concern, as the endogenous RNA-processing machinery might become saturated<sup>159</sup>. In addition, an expression vector (AAV) and direct CNS delivery are required for wide CNS distribution. Regarding neurodegenerative disease, good results have been achieved with RNAi-mediated gene knockdown in Huntington disease<sup>161–163</sup>.

**Genome engineering.**—Another way to reduce the levels of glycogen synthase and/or glycogen-targeting subunits of PPI is to modify the genes that encode these proteins (FIG. 5). The recent development of CRISPR-Cas9 as a biotechnological tool has substantially facilitated genome engineering, and this system is now being harnessed to edit the mammalian genome<sup>164,165</sup>.

A commonly used application of CRISPR-Cas9 is a target gene knockout whereby Cas9 introduces doublestranded DNA breaks (DSBs) adjacent to a guide RNA (sgRNA) recognition sequence, known as a protospacer adjacent motif. DSBs are repaired by non-homologous end-joining (NHEJ), leading to permanent indel formation and a non-functional protein<sup>164–166</sup>. This strategy is applicable to postmitotic cells, such as neurons, in which NHEJ is the preferred DNA repair pathway<sup>167,168</sup>, and the system has been successfully applied for target gene knockout in the mouse brain<sup>169–172</sup>.

CRISPR-Cas9-mediated therapy requires AAV-mediated delivery of Cas9 and sgRNA to the CNS. AAV transduction efficiency again determines which cells express Cas9. In addition, only a proportion of indels lead to biallelic mutation and a non-functional protein, which further decreases the overall efficiency. Other possible downsides include off-target effects, as partial sgRNA sequence similarity might lead to alterations in non-targeted genes, and an immune response provoked by permanently expressed bacterial Cas9 protein.

Technically, correction of laforin or malin at the gene level is possible. A study has shown successful alteration of single DNA bases using the CRISPR-Cas9 system<sup>173</sup>. However, in conditions such as Lafora disease where several different mutations in the associated gene(s) have been identified, base correction therapy would have to be individualized for each patient and would, therefore, be extremely expensive.

**Small-molecule therapies.**—A small-molecule therapy for Lafora disease would provide the least invasive option for the patient, as administration would be in the form of a digestible pill. Small molecules cannot replace the function of laforin or malin at the gene level but could affect the metabolic pathway that leads to the development of Lafora bodies.

For instance, inhibitors of glycogen synthase or the PP1 subunits could prove to be effective therapeutics (FIG. 5).

Identification of small molecules requires high-throughput screening of thousands of molecules and development of in vitro assays to measure the target response. A high-throughput screening assay has been developed to identify small molecules that inhibit glycogen synthase activity to treat adult polyglucosan body disease, a form of GBE deficiency<sup>174</sup>. Inhibitory small molecules were successfully identified using an ex vivo cell-based assay and could potentially provide a therapy for glycogen storage diseases, including Lafora disease. Glycogen synthase inhibition has already been shown to be effective in glycogen storage diseases. Rapamycin, a tuberculosis drug, was found to lower the levels of polyglucosans and glycogen by interfering with the mechanistic target of rapamycin (mTOR) pathway, which is known to be disrupted in other epilepsies<sup>175,176</sup>.

To treat neurological disorders, the BBB usually needs to be crossed, which presents a tremendous challenge. To address this issue, repeated direct and invasive CNS delivery might be required. The target specificity of small molecules is also a potential challenge, and adverse events caused by off-target effects could limit the long-term utility of these therapies.

## Conclusions and future prospects

Common genetic epilepsies tend to be comparatively mild and genetically complex, whereas rare genetic epilepsies are generally severe and genetically simple. The severity of these conditions usually prevents procreation and generational spread, which partially accounts for their rarity. However, until general prenatal or preconceptional screening becomes available, Lafora disease and similar neurodegenerative conditions are likely to persist.

One positive aspect of Lafora disease is its monogenicity, which could permit curative gene replacement in the coming years. Hyperelongation of glycogen chains lies at the root of the disease, and as reducing an activity is generally simpler than replacing a function, therapies aimed at reducing brain glycogen synthesis to mitigate and perhaps reverse the disease are relatively close at hand. Interventions could act at the DNA, RNA or protein level to target a number of enzymes that contribute to glycogen synthesis.

Owing to the considerable progress in Lafora disease research over the past few years, this condition is likely to become treatable before many of the other severe epilepsies. In addition, the insights into laforin and malin function that are emerging from this research are uncovering previously unsuspected roles for these proteins in glycogen metabolism. Further genetic studies in patients with Lafora disease genes are expected to teach us more about the body's main energy store and the bioenergetics of brain function.

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**Key points**

- Lafora disease, a lethal, autosomal recessive, progressive myoclonus epilepsy, is caused by loss-of-function mutations in *EPM2A* or *NHLRC1*, which encode laforin and malin, respectively.
- A large variety of pathogenic variants in *EPM2A* and *NHLRC1* exists, with even distribution across both genes; variants include missense, nonsense and frameshift mutations, as well as larger deletions.
- laforin and malin are implicated in glycogen metabolism and are presented as part of a glycogen quality control mechanism, which decreases the risk of precipitation of individual glycogen molecules.
- In the absence of a functional laforin-malin complex, structurally abnormal glycogen becomes insoluble and accumulates as Lafora bodies, which drive disease progression in the brain.
- The lack of curative treatments and the current understanding of pathogenesis are driving investigations into a variety of therapeutic strategies for Lafora disease, including reduction in brain glycogen synthesis and replacement of the non-functional gene.

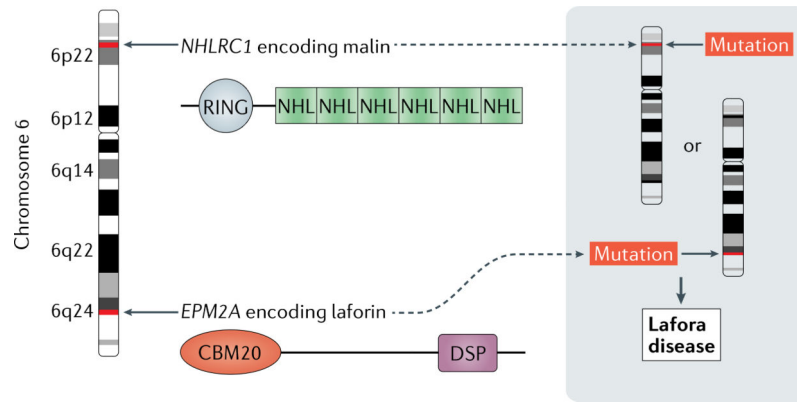
**Box 1 | Lafora disease symptoms**

The first 2–3 years of Lafora disease are characterized by the following symptoms:

- Ataxia
- confusion
- Depression
- Grand mal seizures
- Staring spells and/or absence seizures
- Drop in school performance
- Drop attacks
- Myoclonus
- visual hallucinations
- Headaches
- Dysarthria

**Box 2 | Glycogen metabolism**

Glycogen serves as a cellular store of energy and reduced carbon and is found in many tissues, including the brain. It is a heterogeneous mix of roughly spherical macromolecules, each composed of branched glucan chains with up to 55,000 glucosyl residues per molecule<sup>82</sup>. De novo synthesis of glycogen requires the formation of small glycogen primers. This process usually involves dimers of the protein glycogenin<sup>144</sup>, which mediate first autoglucosylation and then initial chain elongation through formation of  $\alpha$ -1,4 glycosidic linkages<sup>88</sup>. Subsequently, chain elongation is continued by glycogen synthase, which, like glycogenin, uses UDP-glucose as a glucosyl donor. Branching points are introduced by glycogen branching enzyme, which detaches the downstream portion of a glucan chain and reattaches it upstream at the same or a neighbouring chain through an  $\alpha$ -1,6 glycosidic linkage. In the cytosol, glycogen is degraded by glycogen phosphorylase and glycogen debranching enzyme (AGL). Glycogen phosphorylase removes glucosyl units from the end of the glycogen chains but is stalled by branching points, which are cleaved by AGL. Cytosolic glycogen degradation largely yields glucose 1-phosphate, which is available for glycolysis and other cellular pathways<sup>82,88</sup> (FIG. 2c).

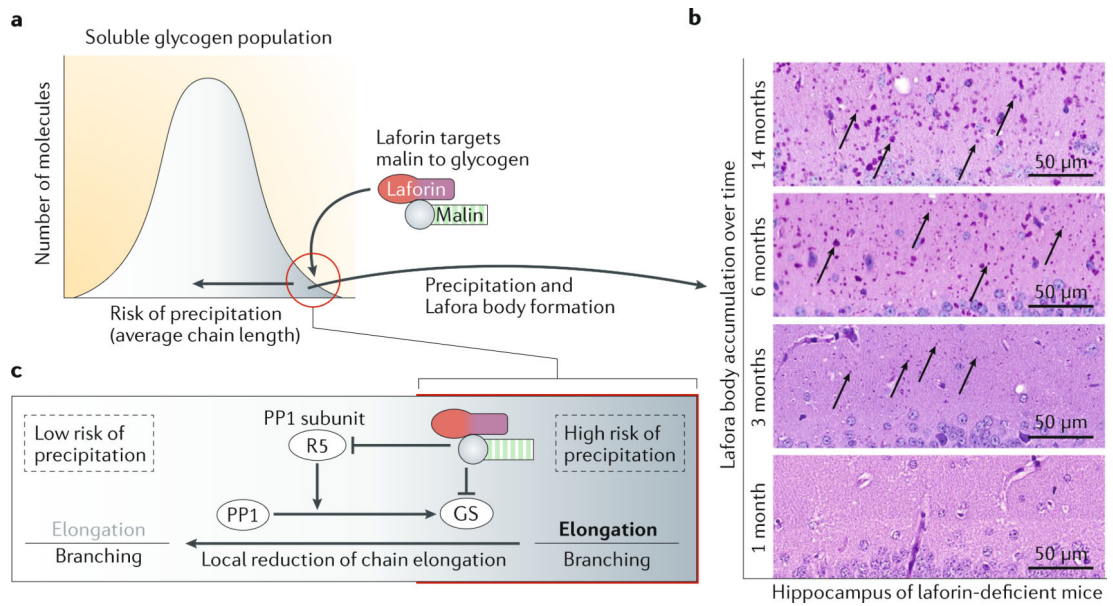


**Fig. 1 |. Causative mutations in Lafora disease.**

*EPM2A* and *NHLRC1* encode laforin and malin, respectively. Both genes are located on chromosome 6. Laforin contains an amino-terminal family 20 carbohydrate-binding module (CBM20) and a carboxy-terminal dual-specificity phosphatase (DSP) domain. Malin contains RING and NHL domains, which are typical for E3 ubiquitin ligases. Mutations — usually missense, nonsense or frameshift — in either of the two genes cause Lafora disease.

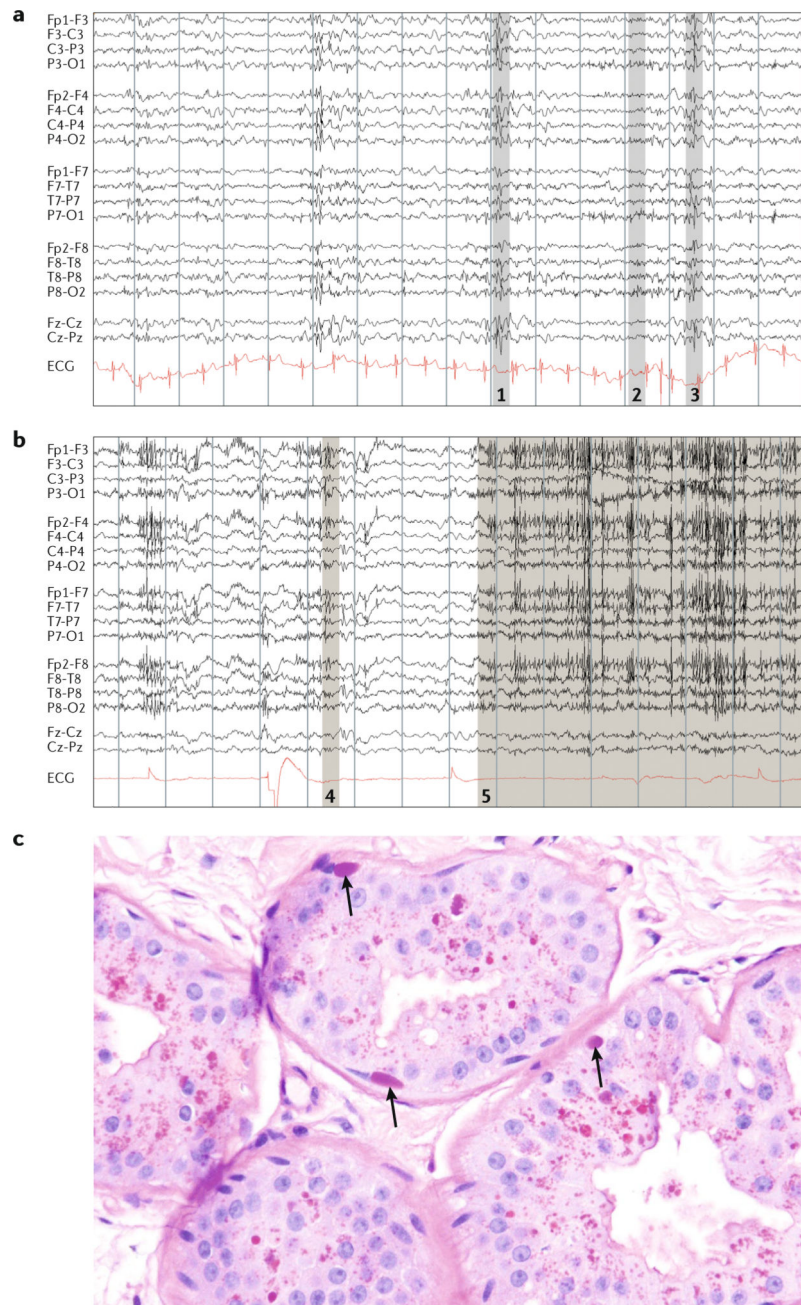






**Fig. 3 | Mechanistic model for Lafora body formation and accumulation.**

**a** | Glycogen is a heterogeneous mixture of molecules that differ in their long glucan chain content and possess different risks of precipitation. To prevent precipitation, a small proportion of molecules that are precipitation-prone (circled in red) might be modified by local reduction of chain elongation, which is achieved by the mechanism explained in part **c**. This modification leads to an increased relative branching frequency and a decreased risk of precipitation (horizontal arrow). **b** | If glycogen precipitation cannot be entirely prevented, precipitated glycogen accumulates over time, as observed in Lafora disease mice of different ages. Arrows indicate some typical Lafora bodies in diastase-resistant periodic acid-Schiff-stained sections of mouse hippocampus. **c** | A functional laforin-malin complex mediates a reduction in glycogen synthase (GS) activity by targeting GS and protein phosphatase 1 (PP1) subunit R5 to degradation, resulting in an increased branching frequency. Rather than affecting the entire GS protein population, this process might occur locally, that is, predominantly on the small proportion of glycogen molecules with a high risk of precipitation (red circle in part **a**). Parts **a** and **c** adapted with permission from REF<sup>87</sup>, CC-BY-4.0.



**Fig. 4 |. Diagnosis of Lafora disease.**

**a** | A 16 s EEG trace in an awake patient with Lafora disease (compound heterozygous for *EPM2A*: Arg241X and Pro211Leu) at age 20 years, 5 years into the disease. Note the slow background rhythms and generalized irregular spike-wave discharges associated with myoclonus. At this age, the patient was still conversant, but each thought and sentence was interrupted by myoclonic absences. **b** | EEG in the same patient at age 28 years in an unresponsive wakeful state. Grey shaded areas in parts **a** and **b** denote patient movements: 1, arm jerk; 2, head jerk; 3, head and arm jerk; 4, twitch; and 5, eyes upward and blinking. **c** | Skin biopsy in the same patient at age 20 years. Arrows indicate Lafora bodies in the

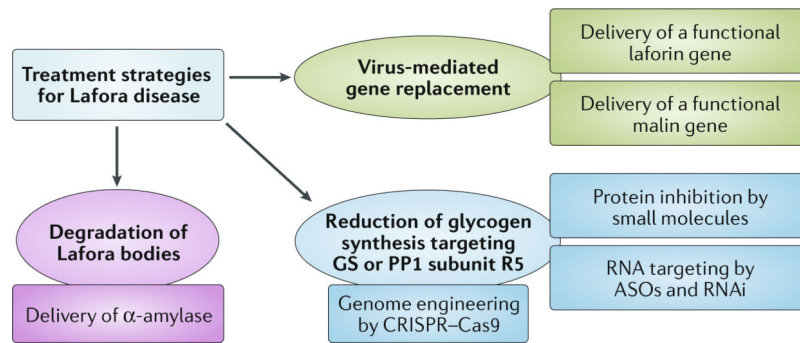
myoepithelium of apocrine glands. The equally stained structures near the lumina of the glands are not Lafora bodies but the normal secretory materials of these cells. Owing to a lack of experience with the disease, many laboratories mistake these materials for Lafora bodies, leading to false-positive diagnoses. ECG, electrocardiogram

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**Fig. 5 |. Overview of therapeutic strategies in Lafora disease.**

In addition to the viral delivery of functional laforin or malin, the therapeutic options for Lafora disease include intracellular degradation of Lafora bodies by glucan-degrading enzymes such as  $\alpha$ -amylase, as well as the downregulation of glycogen synthesis by targeting glycogen synthase (GS) or protein phosphatase 1 (PP1) subunit R5 at the gene, RNA or protein level. ASOs, antisense oligonucleotides; RNAi, RNA interference.