



Lafora's Disease: Towards a Clinical, Pathologic, and Molecular Synthesis

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Lafora's disease is one of five inherited progressive myoclonus epilepsy syndromes. It is an autosomal-recessive disorder with onset in late childhood or adolescence. Characteristic seizures include myoclonic and occipital lobe seizures with visual hallucinations, scotomata, and photoconvulsions. The course of the disease consists of worsening seizures and an inexorable decline in mental and other neurologic functions that result in dementia and death within 10 years of onset. Pathology reveals pathognomonic polyglucosan inclusions that are not seen in any other progressive myoclonus epilepsy. Lafora's disease is one of several neurologic conditions associated with brain polyglucosan bodies. Why Lafora's polyglucosan bodies alone are associated with epilepsy is unknown and is discussed in this article. Up to 80% of patients with Lafora's disease have mutations in the EPM2A gene. Although common mutations are rare, simple genetic tests to identify most mutations have been established. At least one other still-unknown gene causes Lafora's disease. The EPM2A gene codes for the protein laforin, which localizes at the plasma membrane and the rough endoplasmic reticulum and functions as a dual-specificity phosphatase. Work toward establishing the connection between laforin and Lafora's disease polyglucosans is underway, as are attempts to replace it into the central nervous system of patients with Lafora's disease. © 2001 by Elsevier Science Inc. All rights reserved.

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Introduction

Epilepsy (recurrence of unprovoked seizures) occurs in 1% of humans [1] and is the most common neurologic disorder of children. Most epilepsies result from abnormal

neuronal circuitry or excitability, and most seizures can be controlled medically. In 1% of epilepsies the progressive myoclonus epilepsies (PMEs), intractable seizures with a prominence of myoclonic seizures are associated with progressive cortical degeneration after a period of normal brain development [2]. PME's are difficult to diagnose at the first clinical encounter because progression is their defining feature. With the expected advent of gene therapy (human gene therapy trials are presently being organized for several PME's), it is essential to establish rapidly the exact PME diagnosis before excessive central nervous system (CNS) damage. This rapid diagnosis will require a greater mastery of the clinical, as well as the pathologic, features of the PME by the pediatric neurologist (Table 1) [2-7].

The present article focuses on Lafora's disease, an adolescent form of PME with pathognomonic inclusion bodies. In this article, clinical, pathologic, and genetic features are reviewed, and possible pathogenic mechanisms are discussed.

Clinical Features

Age of Onset

In their first decade of life, Lafora's disease patients experience a normal development that cannot be distinguished from those of their unaffected siblings. In the majority of patients the onset of the progressively worsening seizure disorder is between 12 and 17 years of age [7-23], although many patients experience isolated febrile or nonfebrile seizures earlier in childhood [2,13,22,23]. In a minority of patients the progressive syndrome of increasingly intractable seizures can begin as early as 6 years of age [2,23]. In families with more than one affected child, clinical signs, such as subtle myoclonias, visual halluci-

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Table 1. Inherited progressive myoclonus epilepsies

Syndrome	Genes	Age at Onset (year)	Suggestive Clinical Signs	Characteristic Pathologic Features
Neuronal ceroid lipofuscinoses	Eight genes: CLN1-CLN8	0-9, except CLN4 (adult form)	Visual loss resulting from macular degeneration	Lipopigment deposits: granular osmiophilic, curvilinear, or fingerprint profile forms
Unverricht-Lundborg disease	EPM1	6-15	Prominent cerebellar signs; slow progression	None
Lafora's disease	EPM2A*	6-19	Visual hallucinations resulting from occipital seizures	Lafora polyglucosan inclusion bodies
Sialidosis, Type 1	NEU1	8-15	Cherry-red spot	Urinary oligosaccharides, fibroblast neuraminidase deficit
Myoclonic epilepsy with ragged red fibers	MTTK†	3-65	Short stature, lactic acidosis	Ragged red fibers. Mitochondrial respiratory chain abnormalities

* Mutations in at least one other unknown gene also cause Lafora's disease (see text).
† MTTK mutations are responsible in 80-90% of patients. Other cases are due to mutations in the *MTTL1* gene.

nations, or mental decline, are noticed earlier in subsequent affected children than in the proband [23].

Presenting Symptoms

Because of its dramatic nature, most families refer to the first generalized tonic-clonic seizure as the initial symptom. However, any or all of the seizures that will plague the child for the remainder of the course of the disease can be present at onset. These include, in order of importance, myoclonic seizures and occipital seizures with transient blindness, visual hallucinations or photoconvulsion, and atypical absence, atonic, and complex partial seizures [7-23]. Minassian et al. [23] tabulate the presenting clinical features in the first 22 genetically confirmed patients. At or soon after onset, decline in the patient's cognitive skills becomes noticeable [7,13,23].

Clinical Course

The epilepsy that follows consists of increasing occurrence and increasing intractability of the above-mentioned seizures. Status epilepticus with any of the above seizure types becomes common. Antiepileptic drugs are useful, the initial choice of which is valproic acid, which affords coverage for different seizure types. Dysarthria and ataxia appear early, and spasticity, late. Emotional disturbance and confusion are common early in the course of the disease, and dementia sets in gradually [7-23]. Most patients die within 10 years of onset, usually from complications related to degeneration of the nervous system and status epilepticus [13]. Although pathology is not confined to the CNS (see below), non-neurologic symptoms, such as cardiac dysfunction [24], are rare.

Myoclonus and occipital seizures are cardinal components of Lafora's disease. Myoclonus can be fragmentary, symmetric, or massive (generalized). It occurs at rest and is exaggerated by excitement, by action, or by photic stimulation. Both negative (momentary loss of tone) and

positive (jerking) myoclonus have been documented. Myoclonus usually disappears with sleep. Trains of massive myoclonias with relative preservation of consciousness occur, which mimic generalized convulsions [2,13,25]. Myoclonus is the primary reason for early wheelchair dependency; patients in the advanced stages of the disease exhibit continuous generalized myoclonus.

Occipital seizures are common and present as transient blindness, simple or complex visual hallucinations, photomyoclonic or photoconvulsive seizures, or classic migraine with scintillating scotomata [2,13,18]. Prominence of occipital seizures is not a feature of other PME's, such as the neuronal ceroid lipofuscinoses (NCLs). On the other hand, clinically significant retinal pathology is not an important feature of Lafora's disease, although it is an early severe characteristic of the neuronal ceroid lipofuscinoses and sialidosis [8,13].

Neurophysiologic Studies

In our experience and in several reports (see [13]), electroencephalogram (EEG) abnormalities precede clinical symptoms. The EEG background slows, alpha-rhythm and sleep features are lost with progression, and photosensitivity is common. Increasingly, the EEG record becomes replete with paroxysms of generalized irregular spike-wave discharges with occipital predominance and focal, especially occipital, abnormalities [2,18]. Unlike juvenile myoclonic epilepsy and other primarily generalized epilepsies, epileptiform discharges decrease during sleep [2]. The physiologic basis of the myoclonus has been studied extensively and has been demonstrated to be of cortical origin. Photomyoclonus has been demonstrated to proceed by a rapid spread of electrical transients from the occiput to the ipsilateral rolandic area and from there to the contralateral sensorimotor cortex [26,27]. High-voltage somatosensory and visual-evoked potentials are recorded, especially in the early years of the disease. Progressive prolongation of central latencies in the above,

Table 2. Clinical evaluation of Lafora's disease

	At Onset	Later in Disease Course
General physical examination, including liver and spleen sizes	Normal	Normal
Elemental neurologic examination, including fundi and reflexes	Normal	Dysarthria, ataxia, spasticity, fundi remain normal
Mental state examination	Visual hallucinations (epileptic), depressed mood, cognitive deficits	Increased hallucinations, agitation, dementia
MRI of the brain	Normal	Atrophy
EEG	Normal or slow background, occipital spike-wave, photoparoxysmal or photomyoclonic discharges	Slow background, loss of normal sleep features, diffuse focal or generalized spike-waves
Visual-, somatosensory-, and auditory brainstem-evoked potentials	High-voltage visual and somatosensory-evoked potentials	Amplitudes may return to normal size; prolongation of brainstem and central latencies
Nerve conduction studies	Normal	Normal

Abbreviations:
 EEG = Electroencephalogram MRI = Magnetic resonance imaging

as well as in auditory brainstem responses, occur over time [2,16].

Differential Diagnosis and Clinical Evaluation

As summarized in Table 2, the range of ages of onset is wide, and the presenting seizures can be varied [23]. However, most patients present during adolescence with

myoclonic seizures, visual hallucinations, and cognitive difficulties. Although juvenile myoclonic epilepsy may be contemplated, the persistence of EEG background slowing and mental problems should raise the suspicion of a more ominous diagnosis, such as a PME. Magnetic resonance imaging excludes structural abnormalities, and posteriorly dominant irregular spike-wave discharges on EEG (Fig 1) raise suspicion of or generate concerns regarding

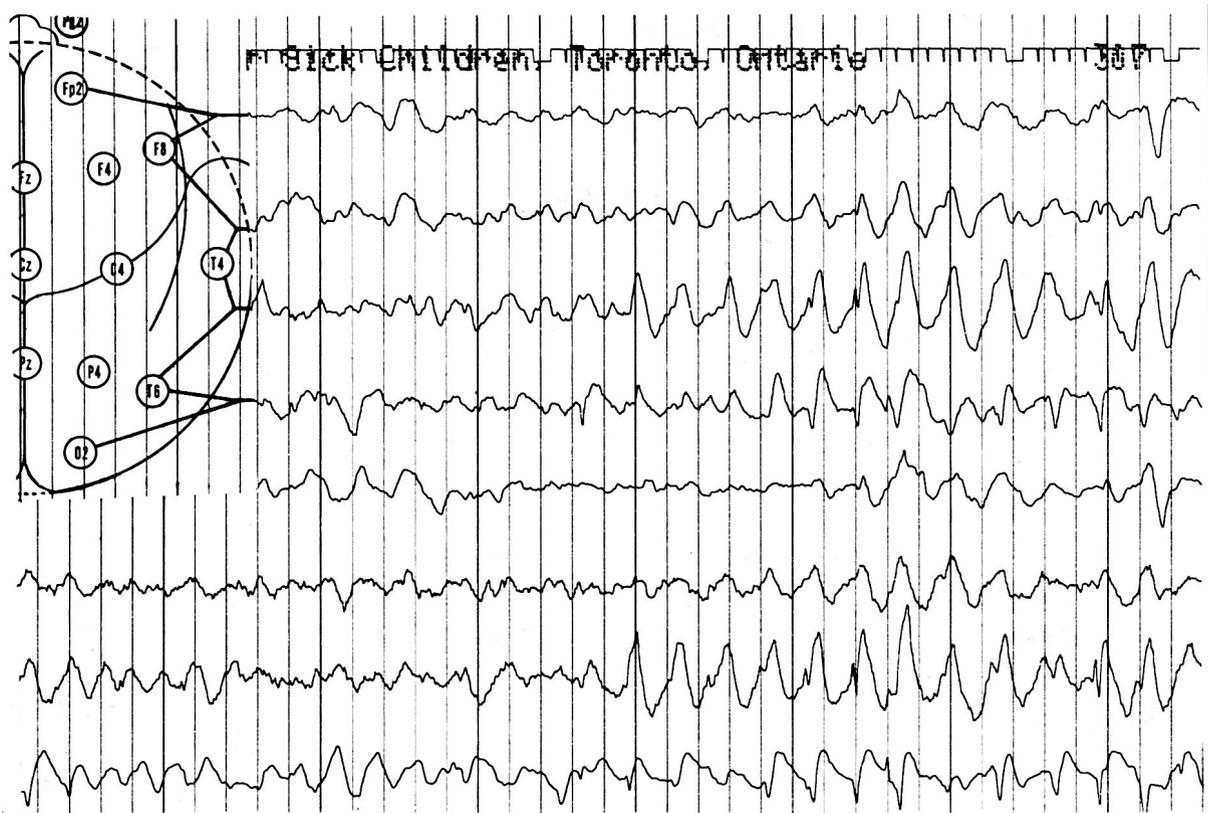


Figure 1. EEG of a 14-year-old female after her first episodes of myoclonia. Lafora's disease is confirmed by mutation detection. The patient's 23-year-old sister is wheelchair bound, has intractable myoclonic seizures and dementia, had lafora bodies in a skin biopsy, and had the same age of onset and mutation as her sister. The last four traces are contralateral to first four.

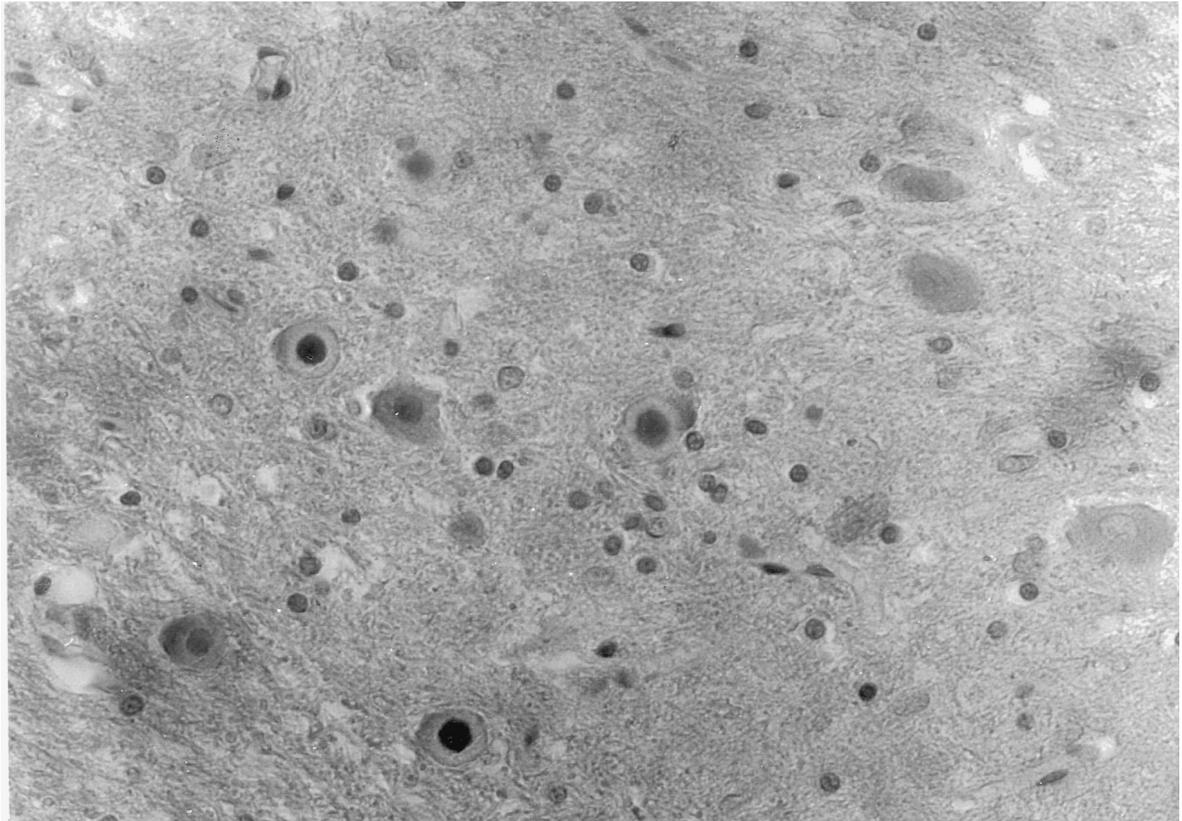


Figure 2. Numerous Lafora bodies with a dark core and paler peripheral zone in dentate nucleus. Hematoxylin-eosin stain. Original magnification $\times 500$.

Lafora's disease. Careful ophthalmologic examination, including electroretinography, is useful in addressing the possibilities of NCLs (in patients presenting early) and sialidosis. Cerebrospinal fluid levels of lactate and titers of measles antibody also can be helpful in dismissing the possibility of myoclonic epilepsy with ragged red fibers and subacute sclerosing panencephalitis, respectively. Although a definitive diagnosis is increasingly moving toward genetic testing (Table 1), a skin biopsy can be equally discriminating and in Lafora's disease will reveal pathognomonic Lafora bodies in most patients (see below).

Pathology

Substantial neuronal loss is observed at postmortem examination. There is no demyelination or inflammation present. All regions of the CNS are involved to varying degrees, including the cerebral and cerebellar cortex, basal ganglia, cerebellar nuclei, thalamus, hippocampus, and retina, as well as anterior and posterior horn cells of the spinal cord [8-14,28].

At brain biopsy, little neuronal loss is observed in earlier phases of the disease despite the presence of significant clinical symptomatology (mainly intractable seizures) [12,14]. This finding indicates that factors in

addition to cortical neuronal loss are likely to be involved in the severe epilepsy of Lafora's disease.

Lafora Bodies

In 1911, Lafora reported striking spherical inclusions in brains of patients with myoclonic epilepsy [28]. Lafora bodies are present primarily in neurons [13,29]. They range in size from 3-40 microns and often occupy the entire cytoplasm. Lafora bodies have a dense core and a less-dense periphery (Fig 2), and they stain strongly positive with periodic-acid Schiff (PAS). PAS staining also reveals numerous small dust-like granules that are composed of the same material as the larger Lafora bodies [8-14,25,28]. The larger Lafora bodies are invariably perikaryal in a distribution similar to the endoplasmic reticulum, and the dust-like granules are concentrated in small neuronal processes (dendrites) [12].

Lafora bodies have been found in other organs, most prominently in liver and muscle [8,15,30-32]. Their shape in non-neural cells usually is not spherical. In skin, they are found in sweat gland duct cells [31] and in apocrine myoepithelial cells [32]. A skin biopsy is the least invasive pathologic method of diagnosis with a very high [31,32] but not perfect [20,33] sensitivity. Specificity of pathologic analysis, however, is close to perfect: PAS-positive inclusion bodies

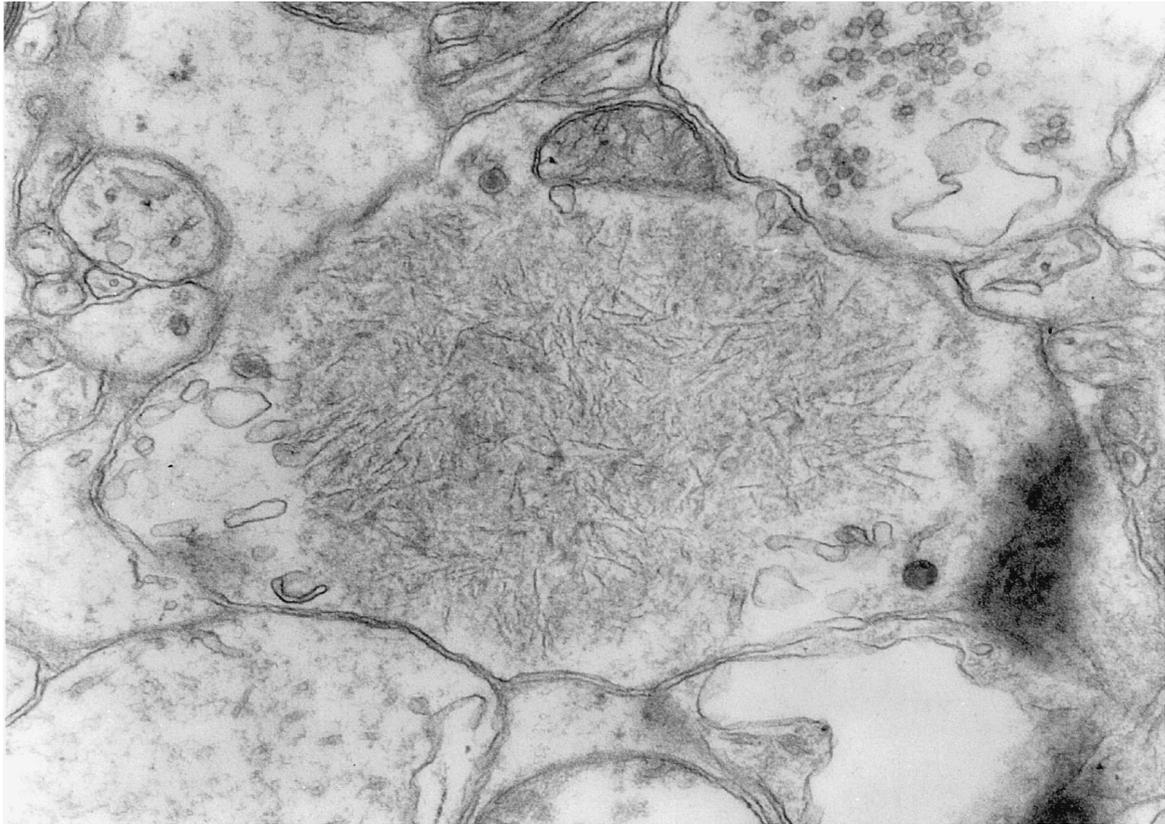


Figure 3. Electron micrograph of a Lafora body in a neuronal process (dendrite); polyglucosan fibrils can be appreciated. Original magnification $\times 30,000$.

in a child between 6 and 18 years of age with myoclonic epilepsy are pathognomonic of Lafora's disease.

Composition of Lafora Bodies

PAS positivity indicates an important content of carbohydrate. Observed under electron microscopy, Lafora bodies are composed of short fibrils of 50-100 Å in diameter and dense granules of 150-300 Å (Fig 3) [8-21, 25,34,35]. The fibrils and granules are packed densely in the core and distributed more loosely in the periphery of the Lafora body. Acid hydrolysis dissolves the fibrils and granules and reveals that they consist almost exclusively of glucose molecules, hence their designation as polyglucosans (glucose polysaccharides) [34,35].

Lafora body-polyglucosans differ from normal glycogen in the following ways: they have shorter branches, are densely packed, insoluble, heavily phosphorylated, and are relatively resistant to digestion with amylases [12,13,34, 35].

Lafora bodies are similar in structure and composition to normal corpora amylacea found in human brains. Corpora amylacea are most prominent in the brains of the elderly, when they can be multitudinous and large. However, they have been observed in nervous systems of patients as young as 10 years of age [29]. Unlike Lafora bodies, corpora amylacea are most prominent in astrocytes

and in the glial feltwork under the ependymal lining of the ventricles and in subpial regions on the surface of the brain. In neurons, they are found exclusively in axons and axon terminals [29].

Lafora bodies are also similar to the polyglucosan bodies found in glycogen storage disease type IV (GSDIV) [36] and adult polyglucosan body disease (APBD) [37]. GSDIV and APBD are allelic disorders caused by different mutations of the glycogen branching enzyme gene [38-40]. GSDIV is a systemic glycogenosis that results in death before 4 years of age from cirrhosis. APBD is characterized by adult-onset progressive sensory and upper and lower motor neuron disease, dementia, but no seizures or myoclonias [37]. Interestingly, the subcellular location of the numerous and large polyglucosan bodies in APBD differs from the location of Lafora bodies in Lafora's disease. APBD inclusions are almost exclusively located in axons or axon hillocks and not in perikarya [37].

Lafora Bodies in Muscle are Within Vesicles

As mentioned, the large Lafora bodies are perikaryally located. In the brain, Lafora bodies are not membrane bound, whereas in muscle and possibly liver, they are surrounded by a membrane [12,15,41]. Based on detailed electron microscopic analysis, Carpenter et al. suggested

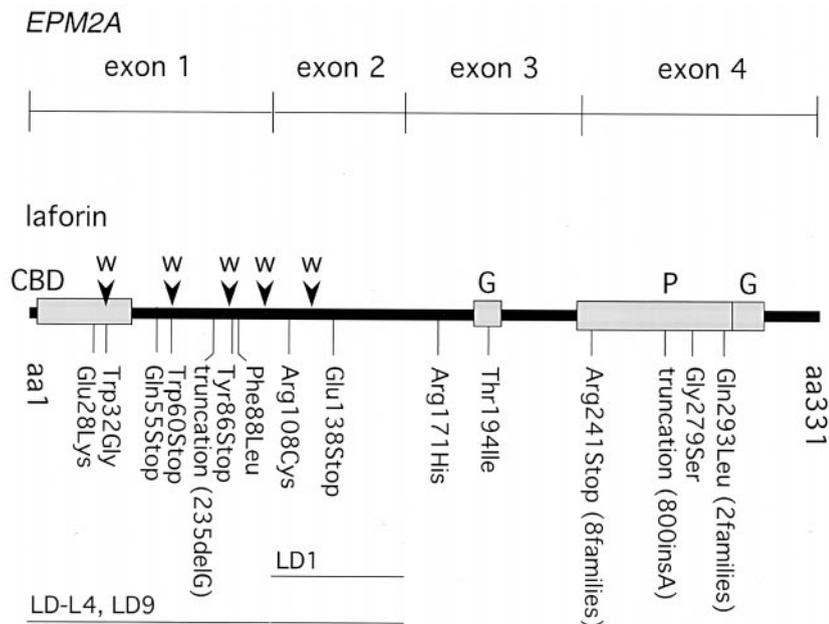


Figure 4. *EPM2A* and laforin. Locations of mutations in our data set of patients are indicated by vertical lines, except for two deletion mutations, which are indicated by horizontal lines; CBD-4, amino acid sequence similarity with carbohydrate binding domain of a number of amylases; G, two potential glucohydrolase domains; P, phosphatase domain; W (arrowheads), positions of tryptophan residues in laforin. Tryptophans placed in semiregular fashion are used by carbohydrate binding proteins for hydrophobic interaction with polysaccharides.

that Lafora body-polyglucosan-containing vesicles are associated closely with the endoplasmic reticulum and are derived from it [12,41]. The vesicles were observed to lack acid phosphatase [10,12] and succinic dehydrogenase [12] activities and therefore are not lysosomes or mitochondria, respectively. They were demonstrated to contain catalase and D-amino oxidase activities, which suggests they may represent early peroxisomes [12].

Genetics and Known Protein Product

Genetics

Lafora's disease is autosomal recessive. At least two chromosomal loci harbor gene mutations that cause Lafora's disease [42,43]. One of those loci is in chromosome 6q24 [42], and the gene responsible for Lafora's disease in this locus, *EPM2A*, has been identified [44]. The second locus remains unknown. Up to 80% of Lafora's disease patients have mutations in the *EPM2A* gene [23,44,45]. No obvious clinical or pathologic differences appear to exist between patients with *EPM2A* mutations and between those whose gene locus does not link to 6q24. The Lafora bodies illustrated in Figures 2 and 3 are from a patient in the latter group and are indistinguishable from other Lafora bodies.

EPM2A is composed of four exons (Fig 4) with several alternative transcripts [44-47]. More than 20 mutations have been reported in all four exons (Fig 4) [23,33,44-48], but so far only three mutations have been found to occur in more than two unrelated families [23]. Despite this extensive mutation heterogeneity, mutation detection assays are available for the detection of all mutations occurring in the coding regions of *EPM2A* [23,33]. Finally, there does not appear to be any correlation between

mutation type and age or seizure types at onset [23]. Correlations between genotypes and clinical course and survival have not yet been performed. *EPM2A* codes for the 331-amino acid protein named *laforin*.

Subcellular Localization of Laforin, the Protein Coded by *EPM2A*

The subcellular localization of laforin recently was determined [49-51]. Laforin associates with the external surface of the rough endoplasmic reticulum and the internal aspect of the plasma membrane [50,51]. Ganesh et al. [49] provided evidence demonstrating that laforin binds to the ribosomes located on the endoplasmic reticulum surface.

Functions of Laforin

Analysis of the sequence of laforin reveals that its C-terminus half contains the consensus amino acid sequence HCxAGxxRS/T found in the catalytic domains of proteins belonging to the protein tyrosine phosphatase family (Fig 4) [23,44]. Members of this large family of more than 500 different proteins are involved in a vast number of biologic pathways and regulate each its target protein by dephosphorylating tyrosine residues. A subfamily of tyrosine phosphatases, the dual-specificity phosphatases, is able to dephosphorylate both tyrosine and serine or threonine residues [53]. Recently laforin was confirmed experimentally to be a dual-specificity phosphatase [49-51]. The same catalytic domain mentioned above is used by one phosphatidylinositol phosphatase [54] and RNA 5'-tri and di-phosphatases [55].

The C-terminus half appears to contain two domains with homology to the catalytic active sites of glucohydrolases (Fig 4) [23]. These glucohydrolases are primarily prokaryotic enzymes, which cleave structural polysaccharides [23,56,57]. Finally, the N-terminus appears to contain a region of homology with the carbohydrate-binding domain of a number of bacterial and lower eukaryotic amylases [23,58,59].

Pathogenic Concepts in Lafora's disease

The two most intriguing questions in Lafora's disease are as follows: What is the origin of the Lafora polyglucosan? Why do Lafora's disease patients have seizures?

Extensive studies have not revealed any significant differences in the constituent polyglucosan in physiologic corpora amylacea, APBD, or Lafora's disease but reveal different cellular locations for corpora amylacea and APBD vs Lafora's disease [12,29,34,35,37,60-62]. It is likely that in all three situations polyglucosans have the same origin but different subsequent migrations.

Regarding corpora amylacea, Cavanagh's recent extensive review states: "corpora amylacea have never been reported within neuronal perikarya at any time in normal subjects" [29]. Neuronal polyglucosan appear to be produced normally in the soma, migrate down the axons where they accumulate and enlarge, and, after the patient reaches 10 years of age, become microscopically detectable [29]. The ultimate fate of these polyglucosan bodies is unknown, although it has been suggested that they transfer to neuroglia [29,63]. With advancing age, corpora amylacea in neuroglia accumulate in large numbers and sizes in the glial feltwork immediately beneath the cerebral ventricles and the pia mater [29]. Passage into the CSF has been postulated as the ultimate route of clearance for corpora amylacea [29].

APBD polyglucosans are also within axons. However, in this case, many of the polyglucosan bodies are large and appear to be stuck in axon hillocks as they migrate down the axon [37]. Although APBD patients have as many polyglucosan bodies as Lafora's disease patients, they do not have epilepsy [37]. Instead they develop upper and lower motor neuron disease and dementia, which is likely secondary to disruption in axonal function [37].

In Lafora's disease, polyglucosan bodies are located in the perikarya and dendrites. Why they do not proceed along the same path as the other polyglucosan is curious. It is possible that laforin plays a role in mediating the movement of the polyglucosan away from the perikaryal region. Laforin's localization at the endoplasmic reticulum and the fact it contains a potential carbohydrate-binding domain would be consistent with such a function.

An alternative possibility is that corpora amylacea and APBD-PG do not originate in the soma but in the axons. It is hard to imagine an exclusively axonal origin for APBD-PG because these polyglucosan clearly originate

from defective glycogen metabolism as a result of mutations of the cytoplasmically expressed glycogen branching enzyme [38-40].

A third possibility is that Lafora's disease-polyglucosan are trapped within or between membranous structures, preventing their flow away from the perikarya. In the brain the many enzymes involved in glycogen synthesis or breakdown are located in the cytoplasm [64] (or on polyribosomes at the external surface of the endoplasmic reticulum in the case of glycogen synthase kinase [65]). Moreover, most investigators agree that neuronal Lafora bodies are not membrane bound [8-14]. On the other hand, Cajal et al. [66] stated that polyglucosan bodies in Lafora's disease, when they are small, are in fact surrounded by a membrane. Carpenter et al. [12] demonstrated that in muscle, the polyglucosan are within endoplasmic reticulum-derived vesicles.

Dendrite and soma membranes determine neuronal excitability. It is possible that the perikaryal and dendritic localization of Lafora bodies may explain the epilepsy in Lafora's disease because of the disturbance to these neuronal compartments after sufficient accumulation of polyglucosans in adolescence.

Other possible functions of laforin may include a role in the digestion of accumulated polyglucosans. This notion is supported by the putative presence of glucohydrolase domains within its primary structure [23]. Laforin may also be involved in regulating one or more enzymes involved in glycogen metabolism. Most dual-specificity phosphatases interact closely with counterpart kinases [67]. A number of dual-specificity kinases localize at the endoplasmic reticulum or ribosomes, including glycogen synthase kinase, and are candidate laforin-interacting proteins. Finally, the role of laforin in the epilepsy of Lafora's disease may be separate from its role in the formation of the Lafora bodies. Our recent detection of laforin at the inner surface of the plasma membrane would be consistent with a potential function of laforin in modifying neuronal excitability.

Much work lies ahead in pursuit of an understanding of Lafora's disease through the avenues revealed by research to date. Identification of the second Lafora's disease gene will be an important step in this process. However, as we move toward a more-complete understanding of this and other PME's, we are also in a position to contemplate cures by replacing the defective gene or protein.

The author would like to dedicate this review to Professor Stirling Carpenter, whose meticulous work in Lafora's disease and adult polyglucosan body disease is a cornerstone in our understanding of these neurologic conditions and their underlying central nervous system physiologies. The author wishes to thank Drs. Stephen W. Scherer and Stirling Carpenter for critically reviewing the manuscript and Drs. Stirling Carpenter and Jacques Thibeault for providing the Lafora body slides (Figs 2 and 3).

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