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Lafora disease, seizures and sugars

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Lafora disease (LD) is the most severe form of Progressive Myoclonus Epilepsy with teenage onset. It has an autosomal recessive mode of inheritance and is almost universally fatal by the second or third decade of life. To date, there is no prevention or cure. In the last decade, with the identification of the genes responsible for this disease, much knowledge has been gained with the potential for the future development of effective treatment. This review will briefly address clinical issues and will focus on the molecular aspects of the disease.

Key words: Lafora disease, progressive myoclonus epilepsy, laforin, malin

Clinical course

Onset is usually between the ages of 12 and 17, although it may occur as early as 6 or as late as 22. The first symptom clearly recognized by the family is usually a generalized tonic-clonic seizure. However, any of the other symptoms or seizures that are seen in LD can occur precede the major convulsion and sometimes go unrecognized for a period of time. Other seizure types include myoclonic seizures, occipital seizures, atypical absence, atonic and complex partial seizures. The seizures may initially respond to anticonvulsants, but they become increasingly resistant to medication (1). Occipital seizures are frequent and can manifest as transient blindness or simple or complex visual hallucinations, although the latter may not be epileptic, and be a psychosis instead (2). Myoclonus can be fragmentary, symmetric or massive, and is usually the primary reason for early wheelchair dependency. Other symptoms that commonly accompany this syndrome include emotional disturbance, depression and confusion early, later evolving into dementia. Nursing and feeding care can alter life span, but it still usually ranges between 17 and 30 years. Recent observations report a slightly longer life span in patients with EPM2B mutations, compared to those with EMP2A (see below). Status epilepticus and aspiration pneumonia are the most common causes of death (1, 3).

Neurophysiology

EEG abnormalities can be seen prior to the clinical symptoms (1, 3). The background activity slows and the normal alpha-rhythm as well as sleep features are lost with disease progression. Photoconvulsive responses are common. Interictal epileptiform abnormalities are seen either with generalized, focal (especially in the occipital regions) or multifocal distribution. Impaired cortical inhibitory mechanisms leading to hyperexcitability are also represented by giant somatosensory and visual-evoked potentials. Progressive prolongation of the central latencies and of brainstem auditory responses are seen during disease evolution (4).

Pathology

Lafora bodies (LBs) are carbohydrate storage products that characterize LD and underlie the epileptic disorder. They are composed of polyglucosans, which are abnormally formed glycogen molecules resembling starch. The polyglucosans in LD consist of long chains of glucose units that are infrequently branched. This makes them insoluble, leading to their accumulation and formation of the LBs (3, 5). LBs stain strongly with periodic acid-Schiff due to their polysaccharide composition, and they are resistant to amylase digestion owing to dense packing (6). Ultrastructural analysis suggests a physical association between newly formed polyglucosans and endoplasmic reticulum (ER) or ER ribosomes (7).

LBs are found in brain, skin, liver, cardiac and skeletal muscle. However, despite this distribution, patients usually do not have extra-neurological manifestations. In skin, LBs are seen in either eccrine sweat gland duct cells or in apocrine sweat gland myoepithelial cells. Skin biopsy can be used for diagnosis if genetic testing is not possible (8). In the central nervous system, LBs are found in the perikarya or dendrites, but not in axons. Perikaryal LBs can grow very large, outgrowing the neuronal body and destroying the cell. However, the total amount of LBs in dendrites exceed the perikaryal accumulation (9). Very large numbers of small dendritic

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LBs in an exceedingly high number of dendrites may play a role in the epileptic diathesis.

Genes

So far, two genes have been identified as causative of LD, namely EMP2A and EPM2B (also known as NHLRC1) (10, 11). The proportion of LD patients with mutations in one or the other gene varies according to the population studied. For instance, one Italian study showed that EMP2A is mutated in 22% and EPM2B in 72% of the patients (12). In our families, EPM2A and EMP2B are mutated in 45% and 43%, respectively. Some biopsy proven LD families do not have mutations in the coding regions of those genes. Linkage and haplotype analysis also excluded linkage to either of the two known genes, suggesting the existence of a third LD locus (13).

Genotype-phenotype correlations

Genotype-phenotype correlations are a challenge at this point. However, some studies have suggested that EPM2B patients have a slower disease progression (12, 14). Another correlation was suggested associating mutations in the first exon of EMP2A to an early onset of cognitive deficit (15).

EMP2A gene is located on chromosome 6q24. It consists of four exons coding for a 331 amino acid protein called laforin (10). Laforin has two isoforms, A and B which localize to the ER and to the nucleus, respectively (16, 17). The isoforms differ in their C-termini, and mutations in the unique isoform A's C-terminus suggests that this is the disease-relevant isoform (17). To date, 40 different mutations and four polymorphisms were identified in this gene (18). These include missense and nonsense mutations, frameshifts and deletions located in the coding region of the gene.

Laforin is a unique protein in that it contains a carbohydrate-binding domain (CBD) of the CBM20 type (19) in its N-terminus and a dual-specificity protein tyrosine phosphatase (DSP) domain in its C-terminus (6, 20). Given the accumulation of polyglycosans in LD and the presence of a CBD, laforin is thought to play an important role in glycogen metabolism (either its synthesis or degradation) (6). Importantly, self-dimerization appears to be necessary for laforin to be functional *in vivo* (21, 22).

Co-immunoprecipitation studies suggest that full-length laforin binds an uncharacterized protein termed EMP2AIP1 (for EPM2A interacting protein). This protein does not appear to be responsible for LD in those LD families with normal EPM2A and EPM2B genes (23). HIRIP5 is another protein shown to interact with laforin. This protein contains a NifU-like domain and a putative MurD ligase domain. However the role of those domains in HIRIP5 function is not yet clear. Interestingly HIRIP5, like laforin, is ubiquitously expressed in subregions of the brain, but predominantly in the cerebellum and hippocampus. This protein also co-localizes with laforin at the subcellular level. Finally, laforin was able to dephosphorylate HIRIP5 on both tyrosine and serine/threonine residues, suggesting that HIRIP5 is a substrate for laforin (24). A third protein shown to interact with laforin, called PTG, is a regulatory subunit of protein phosphatese-1 (PP1) that enhances glycogen accumulation (21). It was shown that the G240S missense mutation identified in some LD patients disrupts the interaction between laforin and PTG (while glycogen binding and phosphatase activity remain preserved). This observation suggests that PTG is critical for laforin function and that laforin is part of a complex of proteins associated with glycogen and may have a role in regulating its metabolism. Studies using a mammalian two-hybrid system demonstrated that laforin interacts with glycogen synthase kinase-3 (GSK3). Furthermore, laforin reduces GSK3 Ser 9 phosphorylation (25, 26). GSK3 is a potent glycogen synthase (GS) inhibitor. The relationship between GSK3, GS, laforin and LBs is discussed below.

EPM2B gene was identified through genome-wide linkage scan followed by haplotype analysis and homozygosity mapping performed in a cluster of French-Canadian families from Quebec (11, 27). To date, 40 mutations have been found in the EMP2B gene, including insertion, missense and nonsense changes, frameshifts and deletions in both compound heterozygous as well as homozygous states. The EMP2B gene product encodes a 395 amino acid protein named malin which contains a zinc finger of the RING type at the N-terminus and six NHL-repeat motifs at the C-terminus. NHL motifs are likely involved in protein-protein interactions, while the RING-finger motif of malin is typical of E3 ubiquitin ligases. Sub-celllular localization studies showed that MYC-tagged malin, similarly to laforin, also localizes to the cytoplasm at the ER and the nucleus (16, 17, 28).

The E3 ubiquitin ligase activity of malin was confirmed in vitro (25, 29). At least two mutations associated with LD (Cys26Ser and Phe33Ser) result in inactivation of malin's ubiquitinase function (13, 25). Ubiquitination can serve several purposes including targeting the ubiquitinated protein for destruction or actively regulating its function (30, 31). Recent studies demonstrated that laforin and malin interact and that this interaction occurs at the central regions of both proteins (25, 29). There is data suggesting that malin ubiquitinates laforin, targeting laforin for destruction, but this is presently difficult to understand, as destruction of laforin by malin would be expected to result in Lafora disease (29). Finally, it was demonstrated through co-immunoprecipitation studies that malin and glycogen synthase (GS) interact, although the result of such interaction is not known.

Animal models of Lafora Disease

Animal models of Lafora Disease known to date include a naturally occurring dog, one transgenic mouse and one knockout mouse.

The canine model was observed in approximately 5% of Miniature Wirehaired Dachshunds (MWHDs) in England. The identified mutation was a dodecamer expansion in the EPM2B gene (32). These animals have a phenotype very similar to the human form of LD, except for the late age of onset (age 6 in dogs, which is equivalent to adulthood in humans).

The transgenic model was created through overexpression of laforin carrying a phosphatase inactivating point mutation (Cys26-6Ser). This dominant-negative model was used to trap the unknown laforin substrate and produce LD pathology. The transgenic LD mouse provided valuable information regarding the laforin protein localization. In brain, this protein localizes to the neuronal soma and dendrites. It was also demonstrated that laforin localizes to the ER, but not to the ribosomes, as initially thought. Importantly, this model allowed the characterization of laforin binding to different polysaccharides, and the preferential binding to polyglucosans over glycogen (33).

The knockout model was created through deletion of the EPM2A exon containing the PTP domain. EPM2A null mice had pathological evidence of LD as early as 2 months of age. Phenotypically, they had normal growth and development until the age of 4 months, when behavioral changes started to occur. Myoclonic seizures, ataxia and electroencephalographic changes were seen at 9 months. These animals showed a peculiar neurodegeneration mechanism involving organellar disintegration. In addition, it was observed that neurodegeneration and the onset of LB inclusions occurred prior to any behavioral abnormalities (34).

Pathophysiology of LBs

To date it is not clear why LBs are formed in the absence of laforin or malin. Evidence to date indicates that both proteins are involved in the metabolism of glycogen. Further supporting this hypothesis is the observation that higher levels of cytosolic glycogen are correlated with higher levels of laforin, while absence of glycogen is correlated with a 60% reduction of laforin. Mouse models which over-express glycogen synthase and have massive over-accumulation of glycogen with lafora-like bodies show a 7-fold elevation of muscle laforin (35). However, laforin binds preferentially to the polyglucosans forming the LBs than to glycogen (33). This observation suggests that polyglucosans do normally form in the cell, likely as a by-product of glycogen metabolism, and laforin's (and possibly malin's) proper function is important in preventing the accumulation of the toxic polyglucosans.

Crafting of properly branched and soluble glycogen requires a number of coordinated enzymatic activities. The complex between these enzymes and glycogen has been termed the glycogenosome. A main component of this complex is glycogen synthase (GS), which elongates glycogen strands by adding glucose units. Glycogen branching enzyme (BE) then moves the extended oligosaccharides to branch points, maintaining the globularity and solubility of glycogen. An abnormally high GS to BE ratio results in inadequately branched polysaccharides, namely polyglucosans (36).

Laforin may downregulate GS via PTG-PP1 and via GSK3. PTG serves to target PP1 to the glycogenosome, where PP1 activates GS by dephosphorylation (37). Laforin binds PTG at PTG's binding site with GS (21). Laforin would therefore downregulate GS by physically outcompeting PTG-PP1 off of GS. GSK3 is the main inhibitor of GS, through phosphorylation of five phosphoregulatory sites on GS (37). Laforin activates GSK3 through dephosphorylation of GSK3 (25, 26). Laforin-activated GSK3 would inactivate GS. In sum, absence of laforin would lead to excess GS activity, GS/BE imbalance, formation of insoluble polyglucosans, and their accumulation into LBs.

The concept of malin and laforin agonistically acting to decrease GS activity in order to promote the right GS/BE balance is in contrast with the observation that malin polyubiquitinates laforin, targeting it for destruction (29). A possible explanation follows: LBs are much more phosphorylated than glycogen, and are in fact more similar to amylopectin than to glycogen. Laforin is able to dephosphorylate amylopectin (38). Therefore, it is possible that laforin could also dephosphorylate LBs, and that the high phosphate content in LBs, compared to normal glycogen, may be a direct consequence of the mutated laforin. Interestingly, glycogen binding appears to inhibit laforin activity (39). Laforin inhibition may be a feedback mechanism to preserve a certain degree of phosphorylation of the glycogen molecule. The role of glycogen dephosphorylation is not clear, but it may be correlated to the maintenance of a properly branched polysaccharide. If laforin activity needs to be kept in check (by glycogen inhibition) to avoid over dephosphorylation of glycogen, it is possible that a mutated malin would lead to lack of ubiquitination and destruction of laforin. Could excess laforin cause such an imbalance of glycogen dephosphorylation to lead to the formation of LBs? Finally, polyglucosans are even more potent inhibitors of laforin DSP activity than normal glycogen. In that case, the initial formation of polyglucosans (either because of mutated laforin, malin or another yet unknown protein) would be aggravated by the further inhibition of any residual laforin activity.

Much information has been gained in LD, but knowledge remains very tentative. Clearly more data are need-

ed to understand the mechanisms causing LD, and maybe then to find a way to make this disease go away.

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