

A SECOND GENE FOR LAFORA DISEASE

Mutations in *NHLRC1* Cause Progressive Myoclonus Epilepsy

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Lafora progressive myoclonus epilepsy is characterized by pathognomonic endoplasmic reticulum (ER)-associated polyglucosan accumulations. We previously discovered that mutations in *EPM2A* cause Lafora disease. We identified a second gene associated with this disease, *NHLRC1* (also called *EPM2B*), which encodes malin, a putative E3 ubiquitin ligase with a RING finger domain and six NHL motifs. Laforin and malin colocalize to the ER, suggesting that they operate in a related pathway protecting against polyglucosan accumulation and epilepsy.

COMMENTARY

Lafora disease is a form of progressive myoclonic epilepsy with onset in late childhood or adolescence. It is characterized by the presence of intracellular polyglucosan inclusions (Lafora bodies) in tissues including the brain, liver, and skin. Patients have progressive neurologic deterioration, which leads to death at an early age. No effective treatment is known for Lafora disease. Inheritance is autosomal recessive, and the first gene, *EPM2A*, was found on chromosome 6q (1). However, mutations in *EPM2A* account for only 75% to 85% of Lafora disease cases (2), so what is causing the disease in the remaining cases? This question has been answered in part by Chan and colleagues, with the discovery of a second gene associated with Lafora disease. Identifying the underlying molecular defects is crucial to developing new therapies for this fatal disorder.

Chan and colleagues set out to identify the second Lafora disease gene by performing linkage mapping on four French-

Canadian families who did not have mutations in *EPM2A*. They identified a locus (*EPM2B*) on chromosome 6p22 (3) and found mutations in a previously unidentified gene, *NHLRC1*. The authors went on to sequence *NHLRC1* in 34 unrelated patients without *EPM2A* mutations. Twenty-six patients were found to have mutations in *NHLRC1*. These patients came from diverse regions including Europe, North and South America, and the Middle East. The mutations were not confined to any specific region of the gene, and they resulted in either single amino acid substitutions or truncation of the protein. The protein produced by *NHLRC1*, called malin by the authors, contains a zinc-binding RING finger and an NHL-repeat domain. The presence of the RING finger suggests an ubiquitin ligase activity, which is associated with targeting proteins for degradation. The NHL-repeat domain is involved in protein–protein interactions. Therefore one possible function of malin is to bind to and target proteins for degradation.

In the families studied by Chan et al., 48% had mutations in *EPM2A*, and 40% had mutations in *NHLRC1*—together accounting for 88% of Lafora disease cases. Therefore at least one other gene causes identical clinical features. Compared with earlier reports, Chan et al. found a lower percentage of *EPM2A* mutations. As more patients are tested for both *EPM2A* and *NHLRC1* mutations, the actual contribution of each gene to causing Lafora disease will become clear. The discovery of genes associated with Lafora disease will aid the understanding of the events that are involved in the formation of Lafora bodies, the onset of seizures, and the subsequent mental decline and death. In the future, gene- or enzyme-replacement therapies for Lafora disease will be developed; in the meantime, the ketogenic diet is being investigated in clinical trials. This is a low-carbohydrate diet, which has the promise of reducing polyglucosan build-up in cells. The identification of Lafora disease genes provides a means of detecting the disease before symptoms appear—an important aspect if diet proves to be an effective therapy.

How do malin and the originally identified Lafora disease gene (*EPM2A*) product, laforin, cause the same clinical features? Chan et al. showed that both malin and laforin localize to the endoplasmic reticulum, which is where the polyglucosan inclusions (Lafora bodies) are found in patients with Lafora disease. Polyglucosan is a heavily phosphorylated, insoluble glycogen-like polymer. The accumulation of polyglucosan in

cells from patients with Lafora disease implies that both laforin and malin are involved in glycogen metabolism. Laforin is a dual-specificity protease and has a carbohydrate-binding domain that has been demonstrated to bind to glycogen (4). Laforin also binds to R5 protein, which targets PP1 phosphatase to glycogen synthase (5). Glycogen synthase, which is activated by dephosphorylation, converts glucose to chains of glycogen. A mouse model overexpressing glycogen synthase was found to develop Lafora bodies (6), and it was suggested that mutations in laforin might result in increased levels of glycogen synthase. Malin is thought to regulate the level of another, as yet unknown protein through ubiquitin-mediated protein degradation. The unknown protein that malin targets for degradation could be glycogen synthase; if so, mutations in malin also would result in increased glycogen synthase activity. An increase in glycogen synthase activity is one relatively simple explanation of how these two different proteins, laforin and malin, could give rise to the same clinical syndrome. The actual pathways involved in the development of Lafora disease, and the relation between malin and laforin, is likely to be far more complicated, involving a complex network of proteins including malin, laforin, and the laforin-interacting proteins EPM2AIP1, HIRIP5, and R5.

The identification of malin, as a second gene associated with Lafora disease, brings us one step closer to understanding the pathogenesis of this progressive myoclonic epilepsy syndrome; however, many questions remain. Does malin directly regulate laforin, glycogen synthase, or another enzyme involved in glycogen metabolism? How do abnormal glycogen metabolism and the subsequent accumulation of polyglucosan lead to seizures? Further studies are required to determine the

precise function of malin and how disruption of this protein leads to Lafora disease.

by Robyn Wallace, Ph.D.

References

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