REVIEW

Phenotype variations in Lafora progressive myoclonus epilepsy: possible involvement of genetic modifiers?

Shweta Singh¹ and Subramaniam Ganesh²

Lafora progressive myoclonus epilepsy, also known as Lafora disease (LD), is the most severe and fatal form of progressive myoclonus epilepsy with its typical onset during the late childhood or early adolescence. LD is characterized by recurrent epileptic seizures and progressive decline in intellectual function. LD can be caused by defects in any of the two known genes and the clinical features of these two genetic groups are almost identical. The past one decade has witnessed considerable success in identifying the LD genes, their mutations, the cellular functions of gene products and on molecular basis of LD. Here, we briefly review the current literature on the phenotype variations, on possible presence of genetic modifiers, and candidate modifiers as targets for therapeutic interventions in LD.

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Progressive myoclonus epilepsies (PMEs) are a group of rare genetic disorders characterized by myoclonic seizures and progressive neurological deterioration.¹ Among the progressive myoclonus epilepsies, Lafora type epilepsy, also known as Lafora disease (LD), is unique because clinical variations are not that common, yet the disease can be caused by defects in any of the three chromosomal loci (6q24, 6p22.3 and one unknown locus).^{1–4} Two genes for LD have already been identified,³ and evidences for the presence of a third locus are ample.^{3,5} Clinical course of the LD is characterized by the onset of myoclonus, grand mal, tonic clonic and absence seizures during the adolescence or in the late childhood. Cognitive decline, dystharrthia and ataxia are noted at 11–17 years.¹ Patients are usually bedridden at around 20 years and then on survival requires respiratory assistance.^{1,6} Pathologically, LD is characterized by the presence of pathognomonic intra-neuronal cytoplasmic inclusions called the Lafora bodies.^{4,6} These inclusions stain positive for the periodic acid-Schiff reagent, suggesting the presence of a large amount of carbohydrate. Biochemical studies demonstrate that these inclusions are made up of abnormally lesser branched and hyperphosphorylated glycogenmoieties.^{7,8} Besides the Lafora bodies, neurodegenerative changes are also seen.⁹ Nonetheless, the cause and consequence of Lafora bodies in LD pathogenesis are yet to be unequivocally established.

The two genes known for LD have been extensively characterized. These are the *EPM2A* and *NHLRC1* genes, coding for a protein phosphatase (named laforin) and an E3 ubiquitin ligase (named malin), respectively.³ Laforin and malin interact with each other and as a complex participate in diverse cellular pathways, including the

glycogen metabolism, ubiquitin–proteasome system and in heat shock response,^{3,10–13} and therefore defects in these processes are thought underlie LD.³ A number of disease-causing mutations, more than 100 of them, are known in *EPM2A* and *NHLRC1*, and several studies have looked at possible genotype–phenotype correlations in the LD families.³ The general consensus is that it would be difficult to ‘predict’ the defective gene during the early stages of LD.³ Indeed, no significant difference in the clinical symptoms was observed in patients harboring mutations in the *EPM2A* gene as compared with those who have had mutations in the *NHLRC1* gene, although the *NHLRC1* patients tend to live longer than the other group.^{14,15} Therefore, it was suggested that malin is one of the upstream regulators of laforin and therefore mutations in malin would affect a few but not all functions of laforin, thus resulting in a milder course of disease.¹⁵ Similarly, an early onset LD phenotype was observed for the *EPM2A* patients with defects affecting the exon-1 of the gene.^{6,16} Here it was suggested that defects in the amino-terminal carbohydrate-binding domain of laforin, mainly coded by the first exon of the *EPM2A* gene, might underlie the early onset phenotype.⁶ Although the proposed models were attractive, subsequent studies have shown that such genotype–phenotype correlations cannot be extended to several other group of patients,^{17,18} suggesting that these models, at best, could be applicable to a few LD families. For examples, several of the *NHLRC1* patients studied showed severe form LD¹⁹ or a late onset LD,²⁰ and not all *EPM2A* patients with exon 1 mutations showed an early onset of the LD symptoms.^{17,18} Intriguingly, a founder effect mutation for *EPM2A* in Arab families showed variable age at onset for LD,²¹ suggesting that a specific

¹Department of Human Biology, International Medical University, Kuala Lumpur, Malaysia and ²Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur, India

Correspondence: Dr S Singh, Department of Human Biology, International Medical University, No. 126, Jalan Jalil Perkasa 19, Bukit Jalil, Kuala Lumpur, WP 57000, Malaysia. E-mail: shweta_singh@imu.edu.my

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Table 1 Interacting partners of laforin and malin and their physiological functions

Interacting partners	Interacts with	Proposed/established cellular functions of the partner	Reference
HIRIP5	Laforin	Laforin interacts with and dephosphorylate HIRIP5; functional significance unknown.	34
EPM2AIP1	Laforin	Interacts with laforin; functional significance unknown.	35
Glycogen synthase kinase 3 (GSK3)	Laforin and malin	Laforin and malin bind to GSK3, and laforin dephosphorylates GSK3; GSK3 is involved in energy metabolism, neuronal cell development and body pattern formation.	36,37
Glycogen-debranching enzyme (AGL)	Malin	Malin interacts with and promotes the ubiquitination of AGL.	38
AMP-activated protein kinase (AMPK)	Laforin and malin	Facilitates malin-mediated degradation of laforin by interacting with and phosphorylating laforin; malin interacts with and ubiquitinate AMPK subunit beta to modulate some unknown function.	10,13,39
Protein targeting to glycogen (PTG/R5)	Laforin–malin complex	PTG is a scaffold protein that binds to glycogen and many of the enzymes and promote glycogen synthesis and the level of PTG is regulated by the malin–laforin complex	10,24,40,41
Heat shock protein 70 (HSP70)	Laforin–malin complex	Laforin, malin and Hsp70 (a cellular chaperone) as a non-redundant partners suppress the cellular toxicity of misfolded proteins.	11
Tau protein	Laforin	Laforin interacts with and dephosphorylate tau, a microtubule-binding protein.	42
Heat shock factor-1 (HSF1)	Laforin–malin complex	Laforin and malin contribute to the activation HSF1—the heat shock-dependent transcription factor, during stress by an unknown mechanism.	12
Carboxyl terminus of Hsp70-interacting protein (CHIP)	Laforin–malin complex	CHIP, a co-chaperone, requires malin–laforin for its translocation to the nucleus upon heat shock and to confer protection against heat stress.	12,43
Neuronatin	Malin	A proteolipid that regulates ion channels during brain development stimulates glycogen synthesis in a malin-dependent way.	44
Dishevelled2	Malin	A key mediator of Wnt signaling pathway, is regulated by malin via its degradation	45

mutation *per se* may not underlie a subsyndrome in LD. Conversely, *NHLRC1* patients from a genetic isolate of tribal Oman with the same mutation showed identical age at onset and similar clinical course of the disease.²² Thus, factors other than the defective *LD* gene could possibly be involved in modifying the disease onset or the progression in some cases. Indeed this has been suggested in an LD family wherein one of the two affected patients presented severe liver failure at onset of LD, although both siblings are homozygotes for the same *EPM2A* mutation.²³

The *LD* gene products laforin and malin are known to interact with a diverse set of proteins and (see Table 1), therefore, variations in gene(s) that code for these interacting protein(s) might contribute to variations in phenotype. Indeed, a recent study demonstrates that a sequence variant in the *PPP1R3C* gene coding for the protein targeting to glycogen (PTG) contributes to a milder course of LD.²³ PTG is one of the glycogen targeting regulatory subunits of protein phosphatase 1 and it positively modulates the affinity of protein phosphatase 1 towards its substrates such as the glycogen synthase and phosphorylase.¹⁰ The laforin–malin complex was shown to negatively regulate the activity of PTG by promoting its degradation via the proteasome.^{10,24} As the loss of laforin or malin results in the genesis of Lafora bodies^{25,26} and suppression of PTG in an LD mouse model rescued, most of the disease symptoms and pathologies,²⁷ it is reasonable to expect that the variant of PTG that associate with the milder phenotype of LD might show a diminished activity towards glycogen synthesis. Biochemical studies have shown that the LD-linked PTG variant indeed displayed decreased ability to promote the glycogen synthesis.²³ Beyond glycogen synthesis, the laforin–malin complex is thought to be involved in other cellular pathways such as endoplasmic reticulum stress,²⁸ clearance of misfolded proteins via the ubiquitin–proteasome pathway or autophagy^{11,29–31} and the heat shock response¹² (see Table 1). It would be therefore important to check possible contributions of sequence variations in critical regulators of these pathways that interact with laforin and/or malin, and test their possible role as modifiers in LD. Given the emerging concept that phenotype variability in patients having the same gene mutation is

dependent on the genetic background and environment,³² and studies have indeed established the contribution of genetic factors other than the disease-causing gene in the phenotype variability,³³ it is imperative to extend such studies to LD as well. Such studies are likely to offer novel “druggable targets” that might help in delaying the onset or the severity of LD if not its treatment.

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