Lafora body disease: NIMHANS experience

P. Satishchandra* and S. Sinha

Department of Neurology, National Institute of Mental Health and Neuro Sciences, Hosur Road, Bangalore 560 029, India

Lafora body disease (LBD) is one of the inherited progressive myoclonus epilepsy syndromes. It is an autosomal-recessive disorder with onset in late childhood or early adolescence. The characteristic features are seizures and progressive deterioration of neurological function. Seizures which include myoclonic and occipital lobe seizures with visual hallucinations and photosensitive seizures. The course of the disease consists of worsening seizures and progressive decline in mental and other neurologic functions that result in death within a decade of its onset. Pathology reveals pathognomonic polyglucosan inclusions that are not seen in any other types of progressive myoclonus epilepsies. Electro physiologically, EEG in LBD reveals slowing of background activity with recurrent epileptiform discharges and photosensitivity. Giant somatosensory evoked potential and visual evoked potential have been documented. LBD is one of the several neurologic disorders associated with brain polyglucosan bodies. Up to 80% of patients with LBD have mutations in the *EPM2A* and *EPM2B* genes. Although common mutations are rare, simple genetic tests to identify these mutations have been established. The *EPM2A* and *EPM2B* genes codes for the protein laforin and malin, which localizes at the plasma membrane and the rough endoplasmic reticulum and functions as a dual-specificity phosphatase. The pathogenetic role of the protein is being established.

Keywords: Lafora body disease, laforin, malin, polyglucason body, progressive myoclonic epilepsy.

Introduction

PROGRESSIVE myoclonic epilepsy (PME) is a syndrome complex that is characterized by the development of progressive myoclonus, cognitive impairment, ataxia and other neurological deficits[1]. It consists of different diagnostic entities and the common causes include Lafora body disease (LBD), neuronal ceroid lipofuscinoses (NCL), Unverricht-Lundborg disease (ULD), myoclonic epilepsy with ragged-red fiber (MERRF) syndrome, sialidosis, dentate-rubro-pallidal-atrophy (DRPLA), storage diseases and some of the inborn errors of metabolism, among others[2-5]. Patients with PME manifest within the first two decades of life and have an autosomal recessive pattern of inheritance. Genetic tests had further enhanced the understanding of the disease process, clarified the features of these disorders and facilitated a rational approach to diagnosis[1-9]. There are few case reports and small series from India about various parameters related to PME[3,6-8,10-13]. However, majority of the large studies related to various subtypes of PME, viz. LBD, NCL are from south India from a single centre, NIMHANS, Bangalore[3,6-8]. Here the research interest in LBD, one of the PMEs, started in the early 1990s.

LBD is a form of progressive myoclonus epilepsy beginning from age 5 to 20 years characterized by generalized tonic-clonic seizures, visual hallucinations (occipital seizures), resting and action (fragmentary) myoclonus, progressive neurological deterioration including ataxia, cognitive and/or behavioural deterioration; Electroencephalogram (EEG) depicting polyspike and wave discharges; basophilic cytoplasmic inclusion bodies present in portions of the brain, the liver and skin as well as the duct cells of the sweat glands. Patients with LBD manifest commonly at 12–14 years of age with autosomal recessive inheritance, regression of acquired milestones, occipital seizures, fragmentary myoclonus and visual impairment with normal retina. They are significantly disabled within a decade of onset. Routine EEG in LBD reveals slowing of background activity with recurrent epileptiform discharges and photosensitivity, which was less commonly observed from India. Giant SSEP and VEP have been documented. Death usually occurs within 10 years of onset; autosomal recessive inheritance and is caused by mutation in the progressive myoclonic epilepsy, 2 gene (*EPM2A*) on chromosome 6q and *NHLRC1* gene. Diagnosis is confirmed by the identification of two mutations in one of the two genes known to be associated with Lafora disease[3,6-8,14-16].

History and epidemiology

LBD (or Lafora disease) is named after Gonzalo Rodriguez Lafora (1887–1971), a Spanish neuropathologist, who reported the presence of spherical inclusions in brains of patients with myoclonus epilepsy, which now known to be called Lafora bodies (Figure 1). These later proved to be the key feature in distinguishing Lafora disease from other myoclonic epilepsies.

The exact figures of prevalence of Lafora disease are not available. It occurs worldwide but because of its autosomal recessive inheritance, it is more frequent in ethnic isolates and in parts of the world with a high rate of
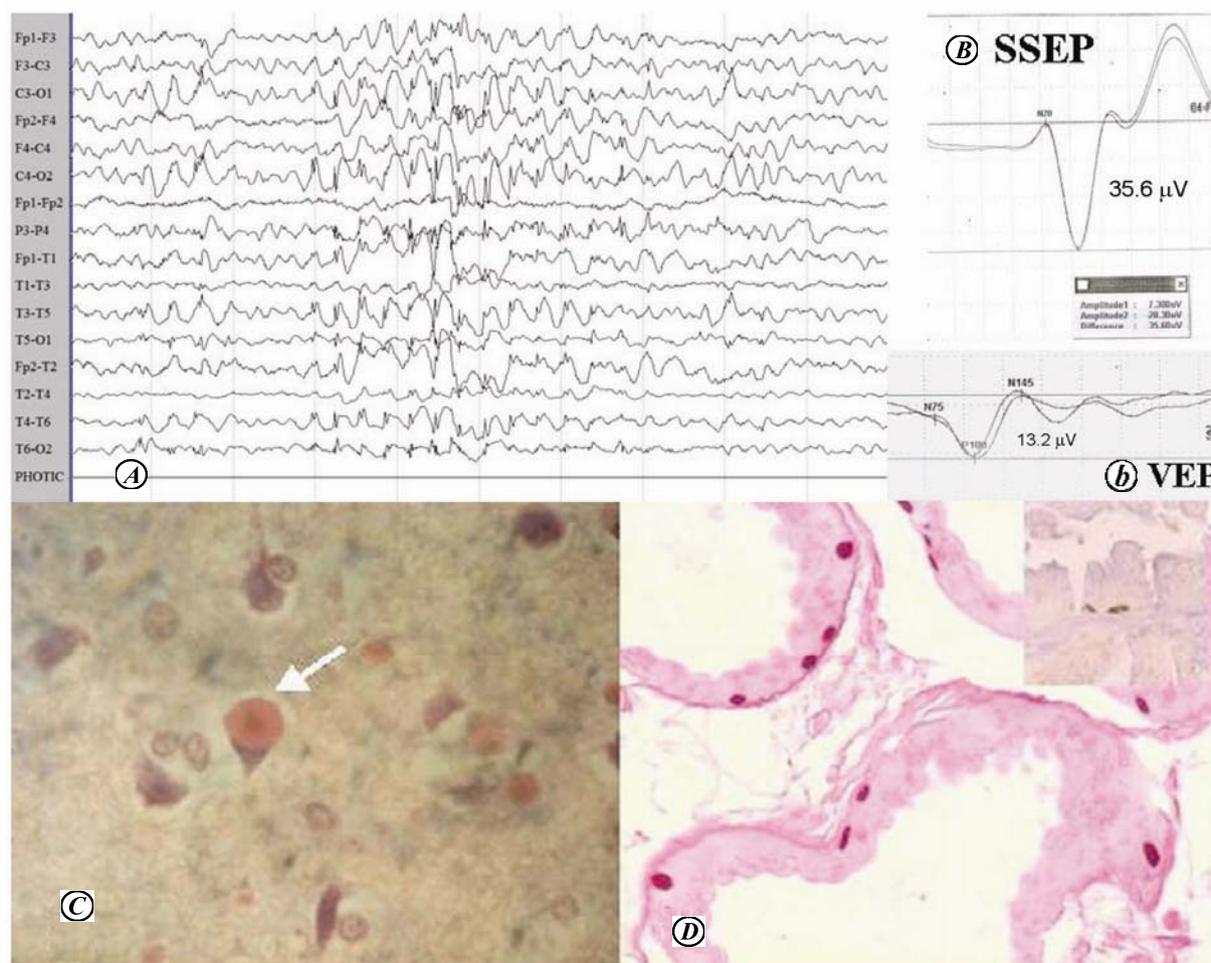
Figure 1 *A–D*. Laboratory parameters showing characteristic features of LBD. *A*, Scalp EEG with diffuse slowing of BGA with frequent spike/polyspike and waves from both the hemisphere; *B*, SSEP: Giant potential (35.6 μV) while recording SSEP from contralateral cortex and stimulating at median nerve; *b*, Giant VEP P100 potential (13.2 μV) during pattern reversal stimulation; *C*, Intraneuronal PAS positive diastase resistant Lafora body in cytoplasm of neurons in the cerebral cortex (X160); *D*, PAS positive diastase resistant Lafora bodies along base of apocrine sweat glands within the myoepithelial cells (X160); Inset: Inclusions are positive for ubiquitin immunostaining (modified from Sinha *et al.*[8]).

consanguinity. Some mutations occur more frequently in specific populations. Examples include the relatively frequent presence of the p.R241X mutation in *EPM2A* in the Spanish population, the p.C26S mutation in *NHLRC1* in French–Canadian families and the p.G158fs16 mutation in *NHLRC1* in tribal Oman. The initial detailed report from India of LBD was by Acharya *et al.*[6]. Subsequently, majority of clinico-pathological reports and pathogenetic and genetic literature on LBD are from NIMHANS, Bangalore and IIT, Kanpur respectively[17].

Clinical manifestations of LBD

There is slight male dominance in our series (M : F :: 24 : 14). In our cohort of patients, history of consanguineous parentage was present in almost three-fourths of patients (73.7%). The mean age at onset of illness was 14.4 ± 3.9 years (range: 10–35 years, median: 14 years) and the mean duration of illness was 2.8 ± 2.1 years. During their first decade of life, patients with LBD have a normal development. In the majority of patients, progressive worsening in seizure occurs between 12 and 17 years of age although some of them would have had few febrile seizures earlier in childhood[18]. In few of them, the progressive syndrome of intractable seizures and myoclonus begin as early as 6 years of age. In families with more than one affected child, the clinical signs like subtle myoclonus, visual hallucinations or mental decline are observed earlier in the subsequent affected children than in the index case. Because of its dramatic nature, most families refer to the first generalized tonic-clonic seizure as the initial symptom. However, other minor seizure types could be the initial manifestation. These include, in order of importance, myoclonic seizures and occipital seizures with transient blindness, visual hallucinations or

photosensitive seizure, atypical absence, atonic and complex partial seizures[19].

In our cohort, generalized tonic-clonic seizure was the manifesting symptom in majority of the patients (71.05%). Myoclonus with or without generalized seizures and progressive cognitive decline were universally present in all the cases. Seizures, myoclonus or learning disability may be the first symptom in majority of the patients[6,8,20]. Seizures are often refractory to anti-epileptic medications. Occipital seizures with visualization of flashes of light (ictal phenomena) were reported in a third of patients while behavioural changes were evident in almost one-fourth of patients in this cohort. Myoclonus is said to be more often fragmentary, asymmetric, arrhythmic and progressively disabling[3,6,21]. Presence of optic atrophy and retinal degeneration has been documented but normal retina is usually noted. Ataxia is often missed because of severe myoclonus. In our cohort, progression from a pre-symptomatic stage to only electrophysiological abnormalities (EEG changes or giant somato-sensory evoked potential (SSEP) potential) and finally to clinically obvious stage have been documented[6,7]. The epilepsy that follows consists of increasing frequency and intractability. Status epilepticus with any of the above seizure types might occur. Myoclonus and occipital seizures are cardinal components of LBD. Myoclonus can be fragmentary, symmetric, or massive (generalized). It occurs at rest and is exaggerated by excitement/action, or photic stimulation. Both negative (momentary loss of tone) and positive (jerking) myoclonus have been documented. Myoclonus usually disappears with sleep. Successive massive myoclonus with relative preservation of consciousness occurs, which mimic generalized convulsions. Myoclonus is often the primary reason for early dependency; patients in the advanced stages have continuous generalized myoclonus. Antiepileptic drugs like valproic acid, etc. are often preferred in view of its broad spectrum.

The disease is subdivided into two types: classical or the Unverricht type and the Lundborg type. The former begins between 6–19 years of age (mean: 11 years). First manifestations could be decreased scholastic performance, behaviour disorders, or seizures. Generalized tonic/clonic and clonic seizures appear initially while myoclonic jerks develop later. Visual deterioration is common. Full blown myoclonic epilepsy develops as the disease progresses. Within 1 to 2 years after the onset of seizures, dementia develops. Death occurs within 2–10 years as a result of heart failure or aspiration pneumonia. The Lundborg type disease has more protracted clinical course. Grand mal seizures are the first manifestation. Myoclonus and dementia are slowly progressive and death usually occurs after the age of 40 years[18,19].

Dysarthria and ataxia appear early, while spasticity is reported late. Emotional disturbance and confusion are common early in the course of the disease and dementia sets in gradually. Most patients die within 10 years of onset, usually from complications related to degenerative process and sometimes due to status epilepticus. Although pathology is not confined to the brain, non-neurologic symptoms, such as cardiac dysfunction, are rare.

One needs to differentiate it often from juvenile NCL and subacute sclerosing panencephalitis (SSPE), especially in some of the atypical cases where periodic slow myoclonic jerks are not evident in SSPE. Occipital seizures are common and present as transient blindness, simple or complex visual hallucinations, photomyoclonic or photoconvulsive seizures, or classic migraine with scintillating scotomata. Prominence of occipital seizures is not a feature of other PMEs like neuronal ceroid lipofuscinoses (NCLs). On the other hand, clinically significant retinal pathology is not an important feature of LBD, although it is an early characteristic of NCL and sialidosis[3,19].

Electrophysiological observations

The characteristic EEG pattern in LBD described is diffuse slowing of background activity with recurrent epileptiform discharges: spikes/polyspikes, with or without slow waves[19]. In our series, scalp EEG ($n = 37$) showed varying degree of slowing of background activity in all but one patient (97.4%) (Figure 1 A). Generalized epileptiform discharges in 84.2% of patients while focal discharges were present in 10 patients. One had multifocal epileptiform activity. Focal discharges have been described in the literature. Photosensitivity with fast frequency stimulus was observed in 5/20 patients only (25%). This phenomenon has been described in the literature[21], but its lesser frequency of occurrence might suggest a different phenotype in India. EEG abnormalities precede clinical symptoms. Pre-symptomatic EEG abnormalities were detected in three of our families[6,7]. Progressive worsening in background activity was observed in four patients. Interestingly, even pre-symptomatic individuals were found to have EEG changes as had been reported for the first time from NIMHANS[7]. The EEG shows slowing of background activity, 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.

Giant SSEP (14 to 175 μV) was demonstrated in 24/31 patients of LBD (Figure 1 B) whereas VEP studies ($n = 31$) revealed a prolonged P100 in 4, absent waveform in 8 and normal in 19 cases. Giant VEP potential have been also documented for the first time from our center[6–8,22] (Figure 1 b). The cause of giant evoked potential is due to enhanced cortical excitability. BAEP studies did not reveal any abnormality. Electrophysiological

features of neuropathy were present in only one patient of LBD[8].

The physiological 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. High-voltage soma-tosensory and visual-evoked potentials are recorded, especially in the early years of the disease. Progressive prolongation of central latencies in the above, as well as in auditory brainstem responses, occurs over time.

Imaging findings

CT ($n = 32$) and MRI ($n = 4$) of brain revealed diffuse cortical atrophy without any parenchymal changes in our cohort. Similar findings have been reported in the literature. Recently, MR spectroscopic abnormalities have been noted in patients with LBD namely reduction in the NAA/creatine ratio and altered NAA/choline and choline/creatine ratios in those without structural MRI to detect brain involvement in frontal cortex, cerebellum and basal ganglia[23,24].

Pathology

In our cohort, in patients with brain biopsies ($n = 2$), the diagnosis of LBD was established by the presence of neuronal intracytoplasmic basophilic, round to oval bodies, which were diastase-resistant PAS positive (Figure 1 C). Brain biopsy for the diagnosis of LBD is now rarely performed. LBD could be easily diagnosed by axillary skin biopsy. The typical inclusions were described initially by Busard et al.[25,26] and later by Berkovic et al.[1]. Similar experience was observed from our centre. The axillary skin biopsies ($n = 35$) revealed characteristic oval to round shaped PAS positive, diastase-resistant Lafora body inclusions in the sweat glands (Figure 1 D). The Lafora bodies were positive for Lugol's iodine and ubiquitin immune-staining (Figure 1 D inset). Histochemically, lafora bodies are polyglucosan and its accumulation could be an error of carbohydrate metabolism. Busard et al. had demonstrated normal pyruvate metabolism in body fluids and brain[25,26].

The 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. 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). 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. 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. 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[19].

Lafora bodies are also similar to the polyglucosan bodies found in glycogen storage disease type IV (GSD IV) and adult polyglucosan body disease (APBD). 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. On the basis of detailed electron microscopic analysis, Carpenter et al.[27] suggested that Lafora body-polyglucosan-containing vesicles are associated closely with the endoplasmic reticulum and are derived from it. The vesicles were observed to lack acid phosphatase and succinic dehydrogenase 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.

Pathogenesis

Studies have not differentiated the constituent of polyglucosan in physiological corpora amylacea, APBD, or LBD but reveal different cellular locations for corpora amylacea and APBD versus LBD. It is likely that polyglucosans have the same origin but different subsequent migrations. Neuronal polyglucosan might be produced normally in the soma, migrate down the axons where they accumulate and enlarge, and, after patient's 10 years of age, become microscopically detectable. With advancing age, corpora amylacea in neuroglia accumulate in large numbers and sizes in the glial feltwork immediately beneath the cerebral ventricles and pia mater. Passage into CSF has been postulated as the ultimate route of clearance for corpora amylacea[19,24,25].

The APBD polyglucosans are located within axons. Although APBD patients have as many polyglucosan bodies as LBD patients, they do not have epilepsy. Instead they develop upper and lower motor neuron disease and dementia, which is likely secondary to disruption in axonal function. In LBD, polyglucosan bodies are located in the perikarya and dendrites. 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. It is also possible that corpora amylacea and APBD-PG originate in the axons. It is difficult to imagine an exclusively axonal origin for APBD-PG because these polyglucosans clearly originate from defective glycogen metabolism as a result of mutations of the cytoplasmically expressed glycogen branching enzyme[19].

The LBD-polyglucosan is trapped within or between membranous structures, preventing their flow away from the perikarya. In the brain, enzymes involved in glycogen metabolism are located in the cytoplasm or on polyribosomes at the external surface of the endoplasmic reticulum in the case of glycogen synthase kinase. Moreover, most investigators agree that neuronal Lafora bodies are not membrane bound. On the other hand, Cajal et al.[28] stated that polyglucosan bodies in LBD, when they are small, are in fact surrounded by a membrane. Carpenter et al.[27] 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 LBD because of the disturbance to these neuronal compartments after sufficient accumulation of polyglucosans in adolescence.

Laforin might be involved in the digestion of accumulated polyglucosans. This is supported by the presence of glucohydrolase domains within its primary structure. Laforin may also be involved in regulating one or more enzymes involved in glycogen metabolism. Most dual-specificity phosphatases interact closely with counterpart kinases. A number of dual-specificity kinases localize at the endoplasmic reticulum or ribosomes, and are candidate laforin-interacting proteins. Finally, the role of laforin in the epilepsy of LBD may be separate from its role in the formation of the Lafora bodies. 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[29].

Genetics

High degree of consanguineous parentage in south India might be responsible for high clustering of the disease in this region. Lafora body disease is autosomal recessive. At least two chromosomal loci harbour gene mutations that cause LBD. One of those loci is in chromosome 6q24, and the gene responsible for LBD in this locus, *EPM2A*, has been identified. Up to 80% of LBD patients have mutations in the *EPM2A* gene. 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 (refs 14–17).

The official name of this gene is 'epilepsy, progressive myoclonus type 2A, Lafora disease (laforin)'. *EPM2A* is the gene's official symbol. The *EPM2A* gene located at 6q24 provides instructions for making a protein called laforin and the initial paper related to it by Minassian et al.[14] included cases from NIMHANS as well. *EPM2A* is composed of four exons with several alternative transcripts. More than 20 mutations have been reported in all four exons, but so far only three mutations have been found to occur in more than two unrelated families. Despite this extensive mutation heterogeneity, mutation detection assays are available for the detection of all mutations occurring in the coding regions of *EPM2A*. Correlations between genotypes and clinical course and survival have not yet been performed. *EPM2A* codes for the 331-amino acid protein named 'laforin'. The subcellular localization of laforin was determined recently. Laforin associates with the external surface of the rough endoplasmic reticulum and the internal aspect of the plasma membrane. Ganesh et al.[17] provided evidence demonstrating that laforin binds to the ribosomes located on the endoplasmic reticulum surface.

Subsequently *NHLRC1* gene (malin) has been discovered. Although laforin protein is active in cells throughout the body, it appears to play a critical role in the survival of nerve cells (neurons) in the brain[11]. Studies suggest that laforin has multiple functions within cells. To carry out these functions, laforin interacts with several other proteins, including malin. These proteins are part of complex networks that transmit chemical signals and break down unneeded or abnormal proteins. Additionally, laforin may act as a tumour suppressor protein, which means that it keeps cells from growing and dividing in an uncontrolled way. Laforin and malin likely play a critical role in regulating the production of a complex sugar called glycogen. Glycogen is a major source of stored energy in the body. The body stores this sugar in the liver and muscles, breaking it down when it is needed for fuel. Researchers believe that laforin and malin may prevent a potentially damaging build up of glycogen in tissues that do not normally store this molecule, such as those of the nervous system. Recently, Rao et al.[30] had suggested that malin is unstable and the aggregate-prone protein and co-chaperone CHIP can modulate its stability and therefore cause cell death. Sharma et al.[31] demonstrated that neuronatin is a novel substrate of malin, which interacts with neuronatin and enhances its degradation through proteasome. Interestingly, neuronatin an aggregate prone protein, forms aggresome upon inhibition of cellular proteasome function and malin recruited to those aggresomes. Neuronatin is found to stimulate the glycogen synthesis through the activation of glycogen synthase and malin prevents neuronatin-induced glycogen synthesis. Their results indicated that malin negatively regulates neuronatin and its loss of function in LBD results in increased accumulation of neuronatin, which might be

Table 1. Identified *EPM2A* and *NHLRC1* mutations in patients with Lafora body disease

Nucleotide change	Predicted effect	Domain affect	Age at onset (year)	Symptoms
EPM2A				
Homozygous c.442A>T	p.N148Y (missense)		11 years	GTCS, myoclonic jerks, cognitive decline, Ataxia, visual disturbances, behavioural problem
Homozygous deletion of exon 3	R159fsX65 (476nu-718nu Ex3)	Exon 3	5 years	Myoclonic jerks
Homozygous deletion of exon	R159fsX65 (476nu-718nu Ex3)	Exon 3	9 years	Cognitive decline, myoclonus jerks
Homozygous deletion of exon	R159fsX65 (476nu_718nuEx3)	Exon 3	NA	NA
Homozygous deletion of exon	R159fsX65 (476un-718n Ex3)	Exon 3	12 years	GTCS, myoclonus
Heterozygous c.28G>A	p.E210K (missense)		17 years	GTCS, myoclonic jerks, congnitive decline, visual disturbances, behavioural problems
Homozygous c.412G>T	p.E138X (nonsense)		NA	NA
NHLRC1				
C64A>C (inferred from mother)	p.S22R (missense)	Close to RING	13 years	Myoclonic jerks, cognitive decline
Homozygous 836T>C	p.L279P (missense)	NHL (4th)	13 years	GTCS, myoclonic jerks
Homozygous c.836T>C	P.l279p (missense)	NHL (4th)	4 years	GTCS, myoclonic jerks
Homozygous c.612ins T	p.F205fs232	NHL(3-6)	NA	NA
Homozygous c.676C>T	p.0226X	NHL(3-6)	16 years	GTCS, myoclonic jerks, cognitive decline, Ataxia, behavioural problem
Homozygous c.377T>C	L126P	NHL(1st)	9 years	GTCS, myoclonic jerks, cognitive decline, visual disturbances, behavioural problems

implicated in the formation of Lafora body or other aspect of disease pathogenesis.

Nearly 100 distinct mutations have been discovered in the two genes in over 200 independent LD families. Nearly half of them are missense mutations and the deletion mutations account for one-quarter. Defects in at least three genes underlie LD, of which two have been isolated and their mutations characterized: The *EPM2A* gene (MIM# 607566) encoding laforin[14–17,29–36] and the *NHLRC1* gene (MIM# 608072) encoding malin[15]. Laforin is a protein phosphatase, which is ubiquitinated by malin before degradation[14–17,29–36]. Aberrant functions of laforin and/or malin, which eventually affect the post-translational modification of target proteins, are likely to underlie the onset and progression of LBD[11,32]. Almost all the work related to the genetics of LBD in India has been carried out by Ganesh's team at IIT Kanpur with patients from NIMHANS (Table 1).

Mouse models in Lafora body disease

Puri *et al.*[37] studied the laforin-deficient mice as a model and showed that Lafora bodies recruit proteasomal subunit, endoplasmic reticulum chaperone GRP78/Bip, autophagic protein p62 and endosomal regulators Rab5 and Rab7. The laforin-deficient brain also revealed proliferation of enlarged lysosomes, lipofuscin granules, amyloid-β peptides and increased levels of insoluble form of ubiquitinated protein, indicating a significant impairment in the cellular degradative pathway. Further, abnormal dendrites and increased gliosis, especially at the vicinity

of Lafora bodies, were noted in the LBD brain. They suggested that the neuropathology in LBD is not limited to Lafora bodies and some of the neuropathological changes in LBD are likely to be secondary effects caused by Lafora bodies and that impairment in the autophagy-endosomal-lysosomal pathways might underlie some of the symptoms in LD. In another study by Puri *et al.*[38], from the same group on the *EPM2A* gene knock-out mice, revealed presence of hyperphosphorylated Tau protein in the brain. Intriguingly, NFTs were also observed in the skeletal muscle tissues of the knock-out mice. The hyperphosphorylation of Tau was associated with increased levels of the active form of GSK3 beta. The observations on Tau protein were replicated in cell lines using laforin overexpression and knockdown approaches. They suggested that laforin is one of the critical regulators of Tau protein, that the NFTs could underlie some of the symptoms seen in LD and that laforin can contribute to the NFT formation in Alzheimer's disease and other tauopathies.

1. Berkovic, S. F., Andermann, F., Carpenter, S. and Wolfe, L. S., Progressive myoclonus epilepsies: specific causes and diagnosis. *New Engl. J. Med.*, 1986, **315**, 296–305.
2. Berkovic, S. F., Cochius, J., Andermann, E. and Andermann, F., Progressive myoclonic epilepsies: clinical and genetic aspects. *Epilepsia*, 1993, **34**, 19–30.
3. Satishchandra, P. and Sinha, S., Progressive myoclonic epilepsy: Indian perspective. *Neurology India*, 2010, **58**, 514–522.
4. Lake, B. D., Lysosomal and peroxisomal disorders. In *Greenfield's Neuropathology* (eds Adams, J. H. and Duchen, L. W.), Edward Arnold, London, 1997, 6th edn, pp. 668–676.

5. Marseille Consensus Group. Classification of progressive myoclonic epilepsies and related disorders. *Ann. Neurol.*, 1990, **28**, 113–116.

6. Acharya, J. N., Satishchandra, P., Asha, T. and Shankar, S. K., LBD in south India – a clinical, electrophysiological and pathological study. *Epilepsia*, 1993, **34**, 476–487.

7. Acharya, J. N., Satishchandra, P. and Shankar, S. K., Familial progressive myoclonic epilepsy: clinical and electrophysiologic observations. *Epilepsia*, 1995, **36**, 429–434.

8. Sinha, S., Satishchandra, P., Yasha, T., Gayathri, N. and Shankar, S. K., Progressive myoclonic epilepsy: a clinical, electrophysiological and pathological study from south India. *J. Neurol. Sci.*, 2007, **252**, 16–23.

9. Shahwan, A., Farrell, M. and Delanty, N., Progressive myoclonic epilepsies: a review of genetic and therapeutic aspects. *Lancet Neurol.*, 2005, **4**, 239–248.

10. Singh, S. *et al.*, Lafora progressive myoclonus epilepsy: a meta-analysis of reported mutations in the first decade following the discovery of the *EPM2A* and *NHLRC1* genes. *Hum. Mutat.*, 2009, **30**, 715–723.

11. Dastur, D. K., Singhal, B. S., Gootz, M. and Seitelberger, M., Atypical inclusion bodies with myoclonic epilepsy. *Acta Neuropathol.*, 1966, **7**, 16–25.

12. Chowdhury, C., Jain, S., Roy, S. and Maheshwari, M. C., LBD: a case report. *Neurology India*, 1984, **32**, 59–64.

13. Mehndiratta, M. M., Agarwal, P., Tatke, M. and Krishnamurthy, M., Neurological mitochondrial cytopathies. *Neurol. India*, 2002, **50**, 162–167.

14. Minassian, B. A. *et al.*, Mutations in a gene encoding a novel protein tyrosine phosphatase cause progressive myoclonic epilepsy. *Nature Genet.*, 1998, **20**, 171–174.

15. Chan, E. M. *et al.*, Mutations in NHLRC1 cause progressive myoclonus epilepsy. *Nature Genet.*, 2003, **35**, 125–127.

16. Ianzano, L., Zhang, J., Chan, E. M., Zhao, X. C., Lohi, H., Scherer, S. W. and Minassian, B. A., Lafora progressive Myoclonus epilepsy mutation database – *EPM2A* and *NHLRC1* (*EPM2B*) genes. *Hum. Mutat.*, 2005, **26**, 397.

17. Ganesh, S., Puri, R., Singh, S., Mittal, S. and Dubey, D., Recent advances in the molecular basis of Lafora's progressive myoclonus epilepsy. *J. Hum. Genet.*, 2006, **51**, 1–8.

18. Genton, P., Borg, M., Vigliano, P., Pellisier, J. and Roger, J., Semi-late and rapidly progressive case of LBD with predominant cognitive symptoms. *European Neurol.*, 1989, **29**, 333–338.

19. Delgado-Escueta, A. V., Advances in lafora progressive myoclonus epilepsy. *Curr. Neurol. Neurosci. Rep.*, 2007, **7**, 428–433.

20. Rapin, I., Myoclonus in neuronal storage and Lafora diseases. In *Myoclonus – Advances in Neurology* (eds Fahn, S., Marsden, C. D. and Von Woert, M. H.), Raven Press, New York, 1986, vol. 43, pp. 65–85.

21. van Heycop ten Ham, M. W., Lafora disease: a form of progressive myoclonic epilepsy. In *Handbook of Clinical Neurology* (eds Vinken, P. J. and Bruyn, G. W.), North Holland Publishers, Amsterdam, 1974, vol. 15, pp. 382–422.

22. Canafoglia, L. *et al.*, Sensorimotor cortex excitability in Unverricht–Lundborg disease and Lafora body disease. *Neurology*, 2004, **63**, 2309–2315.

23. Villanueva, V. *et al.*, MRI volumetry and proton MR spectroscopy of the brain in Lafora disease. *Epilepsia*, 2006, **47**, 788–792.

24. Altindag, E., Kara, B., Baykan, B., Terzibasioglu, E., Sencer, S., Onat, L. and Sirvanci, M., MR spectroscopy findings in Lafora disease. *Neuroimaging*, 2009, **19**, 359–365.

25. Busard, B. L. S. M., Renier, W. O., Gabreels, F. J. M., Jaspar, H. H. J., Van Haelst, U. J. G. and Sloof, J. L., LBD: comparison of inclusion bodies in skin and in brain. *Arch. Neurol.*, 1986, **43**, 296–299.

26. Busard, B. L. S. M., Gabreel-Festen, A. A. W. M., Renier, W. O., Gabreels, F. J. M. and Stadhouders, A. M., Axillary skin biopsy: a reliable test for the diagnosis of LBD. *Ann. Neurol.*, 1987, **21**, 599–601.

27. Carpenter, S., Karpati, G., Andermann, F., Jacob, J. C. and Andermann, E., Lafora's disease: peroxisomal storage in skeletal muscle. *Neurology*, 1974, **24**, 531–538.

28. Rodríguez-Somoza, R., Lafora and the Instituto Cajal. *Arch. Neurobiol. (Madr).*, 1965, **28**, 340–352.

29. Serratosa, J. M., Gardiner, R. M., Lehesjoki, A. E., Pennacchio, L. A. and Myers, R. M., The molecular genetic bases of the progressive myoclonus epilepsies. *Adv. Neurol.*, 1999, **79**, 383–398.

30. Rao, S. N., Sharma, J., Maity, R. and Jana, N. R., Co-chaperone CHIP stabilizes aggregate-prone malin, a ubiquitin ligase mutated in Lafora disease. *J. Biol. Chem.*, 2010, **285**, 1404–1413.

31. Sharma, J., Rao, S. N., Shankar, S. K., Satishchandra, P. and Jana, N. R., Lafora disease ubiquitin ligase malin promotes proteasomal degradation of neuronatin and regulates glycogen synthesis. *Neurobiol. Dis.*, 2011, **44**, 133–141.

32. Mittal, S., Dubey, D., Yamakawa, K. and Ganesh, S., Lafora disease proteins malin and laforin are recruited to aggresomes in response to proteasome impairment. *Hum. Mol. Genet.*, 2007, **16**, 753–762.

33. Gentry, M. S., Worby, C. A. and Dixon, J. E., Insights into Lafora disease: malin is an E3 ubiquitin ligase that ubiquitinates and promotes the degradation of laforin. *Proc. Natl. Acad. Sci. USA*, 2005, **102**, 8501–8506.

34. Lohi, H. *et al.*, Novel glycogen synthase kinase 3 and ubiquitination pathways in progressive myoclonus epilepsy. *Hum. Mol. Genet.*, 2005, **14**, 2727–2736.

35. Swick, H. M., Diseases of gray matter. In *Pediatric Neurology* (ed. Swaiman, K. F.), Mosby, USA, 1988, vol. 2, 1st edn, pp. 772–794.

36. Ceuterick, C. and Martin, J. J., Diagnostic role of skin or conjunctiva biopsies in neurological disorders. *J. Neurol. Sci.*, 1984, **65**, 170–191.

37. Puri, R., Suzuki, T., Yamakawa, K. and Ganesh, S., Dysfunctions in endosomal-lysosomal and autophagy pathways underlie neuropathology in a mouse model for Lafora disease. *Hum. Mol. Genet.*, 2012, **21**, 175–184.

38. Puri, R., Suzuki, T., Yamakawa, K. and Ganesh, S., Hyperphosphorylation and aggregation of Tau in laforin-deficient mice, an animal model for Lafora disease. *J. Biol. Chem.*, 2009, **284**, 22657–22663.