DIAGNOSIS AND SYMPTOMATOLOGY OF LAFORA'S DISEASE

FINAL DEGREE PROJECT - JUNE 2019

Main area: Physiology and physiopathology
Secondary areas: Biochemistry and Public Health

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1. ACRONYMS

AMRF: Action myoclonus-renal failure syndrome.

CNS: Central nervous system

DRPLA: Dentatorubral-pallidoluysian atrophy.

EPM1: Unverricht-Lundborg disease.
EEG: Electroencephalogram.
EPM4: Action myoclonus-renal failure syndrome.
EPM5: PME-ataxia syndrome.
EPM6: North Sea PME.

FDA: Food and Drug Administration.

GS: Glycogen synthase

IGE: Idiopathic generalized epilepsy

JME: Juvenile myoclonic epilepsy.

LD: Lafora Disease.
LB(s): Lafora body(ies).

MERRF: Myoclonus epilepsy with ragged-red fibers.
MEAK: Myoclonus epilepsy and ataxia due to pathogenic variants in the potassium channel.
MRI: Magnetic resonance imaging.

NCBI: National center for biotechnology information.
NLM: National library of medicine.
NCL: Neuronal ceroid lipofuscinoses.

PME(s): Progressive myoclonic epilepsy(ies).

ROS: Reactive oxygen species

2. ABSTRACT

Lafora disease (LD) is a rare and neurodegenerative illness, which is classified within the group of progressive myoclonic epilepsies (PMEs) and is initially presented from 6 to 20 years of age. Today neither a diagnosis prior to the onset nor an effective treatment are available. The 100% of patients have a fatal end approximately within 10 years after manifestations' start. The aim of this study is to analyze physiopathological basis of the disease, and the diagnostic strategies followed nowadays. Also, an informative diptych and an algorithm that might provide support to diagnosis by non-specialized professionals, have been proposed.

In the present work it has been concluded that LD is an autosomal recessive disease, caused by dysfunctional mutations in the gene EPM2A or NHLRC1 generally. These codify for the complex's proteins Laforin-Malin, whose dysfunction promotes glycogen precipitation into Lafora Bodies (LBs), the pathogenic cause. This disease is heterogeneous genetically, but homogeneous phenotypically, although there are pathogenic variants of its progression, as *mild LD* and *early-onset LD*. Its low incidence implies unknowledge between professionals and a complicated diagnosis, which requires three levels of evidence: clinical manifestations' presence; electroencephalogram (EEG) and LB's observation; and finally, genetic confirmation. Some tests as EEG and axillary skin biopsy might be useful for early diagnosis, so as to establish a preventive treatment. Summarizing, the lack of experimental samples and experts difficults the investigation, but we are moving towards effective treatment.

RESUM

La malaltia de Lafora (*Lafora Disease*, LD) és una malaltia rara i neurodegenerativa que es troba dins el grup d'epilèpsies mioclòniques progressives (PMEs) i que es presenta inicialment des dels 6 als 20 anys d'edat. Actualment no es disposa d'un diagnòstic previ a l'inici ni d'un tractament efectiu. El 100% dels pacients tenen un final fatal transcorreguts aproximadament 10 anys des de l'inici clínic. Aquest estudi té l'objectiu d'analitzar la seva base fisiopatològica i les estratègies diagnòstiques seguides actualment. A més, s'ha proposat un díptic informatiu per a la població, i també un algoritme que podria facilitar un diagnòstic als professionals no especialitzats.

Aquest treball conclou que la LD és autosòmica recessiva, causada per mutacions afuncionals als gens EPM2A o NHLRC1, generalment. Aquests codifiquen per les proteïnes del complex Laforina-Malina, la disfunció del qual provoca la precipitació del glicogen en *cossos de Làfora* (LBs), la causa patogènica. La malaltia és heterogènia genèticament, però homogènia fenotípicament, tot i així, hi ha variants patogèniques de la progressió, com la *LD benigna* o la *LD d'inici prematur*. La seva baixa incidència implica desconeixença dels professionals i un diagnòstic complicat, que requereix tres nivells d'evidència: presència de característiques clíniques; alteracions en l'electroencefalograma (EEG) i l'observació d'LBs; i finalment la confirmació genètica. Algunes proves com l'EEG i la biòpsia de pell axilar podrien servir de diagnòstic precoç per establir un tractament preventiu. En resum, la investigació es troba dificultada per l'escassetat de mostres experimentals i d'experts, però és el passatge cap a un tractament eficaç.

3. INTEGRATION FIELD

Neurodegenerative diseases progressing to dementia are a big concern to the aging society. They affect mainly the elderly, but this final degree project is addressed to one of the exceptions to those progressively incapacitating affections: Lafora disease. It is a rare illness which afflicts children and young teenagers, starting from a progressive myoclonic epilepsy and leading them to a fatal end.

Physiology and physiopathology are two medical disciplines. The first studies the healthy functioning of a living being and the second deals with the damaged physiological system that cause, is associated to or results from an illness; with the intention to explain functional changes in the organism due to that pathological state (1). As the main objectives are to describe the symptomatology and diagnostic strategies derived from Lafora disease, the principal area of study is physiology and physiopathology.

Biochemistry is the discipline that describes the molecular processes or mechanisms that take place in the organism, describing either usual or abnormal conditions observed during the disease progression. It has been integrated as a secondary area of study in this project because it is essential to understand firstly the molecular mechanisms causative of the illness' symptoms, as well as to comprehend how new treatment approaches could act. Therefore, also Pharmacology is slightly treated, as general treatment strategies are explained.

Finally, the other secondary area of study implied is Public Health. It is not only a study field, it is considered a science and a discipline for preventing diseases, prolonging life and promoting human health through the information of the society (2). The epidemiology and the geographical distribution of Lafora's disease is described below. Moreover, despite its little incidence, it has a great impact, both on the patients and their families, and on health expenses. Due to the lack of awareness involving a rare disease, this project aims to briefly disclose Lafora disease, as it may give a ride to its investigation and progress of treatment strategies.

4. INTRODUCTION

4.1. PROGRESSIVE MYOCLONUS EPILEPSIES

Progressive Myoclonus Epilepsies (PMEs) are severe and very disabling diseases, with usually fatal outcome. Diagnosis is complicated because most patients are healthy before the onset of the neurological decline. Although we are currently beginning to understand the gene defects, there is still a long way to go because these errors do not always explain the full pathogenesis (3).

PMEs are rare and heterogeneous disorders. They are among the most disabling forms of epilepsy, combining myoclonus, epileptic seizures and progressive neurologic deterioration (4,5).

Particularly, the occurrence of myoclonus is asymmetric, asynchronous and arrhythmic, and has a focal or segmental distribution. Epilepsy predominates in tonic-clonic seizures, but other types can also be shown, as absence, tonic or focal seizures. Finally, the neurologic decline may be characterized by ataxia, neuropathy, myopathy and what's more noticeable, progressive cognitive decline. But the progress and the prognosis of the disease might vary, as depends on the specific form of PME.

The nature and limits of the PMEs were described in the Marseille meeting (5), where a consensus group formed by researchers and clinicians classified PMEs taking into account the criteria of its clinical entities and the description of markers that lead to a precise diagnosis of each, as pathological, biological and neurophysiological characters.

The main PMEs are Lafora disease (LD), Neuronal ceroid lipofuscinoses (NCL), Type 3 neuronopathic Gaucher disease, Unverricht-Lundborg disease (EPM1), Sialidosis, Myoclonus epilepsy and ragged-red fibers (MERRF), Dentatorubral-pallidoluysian atrophy, Action myoclonus-renal failure syndrome (AMRF; EPM4), PME-ataxia syndrome (EPM5) and North Sea PME (EPM6).

In contrast to the highest incidence PMEs, the EPM1, which has a slow progression and whose patients can achieve a normal life span, LD, NCL and the neuropathic form of Gaucher disease have an unfailingly fatal outcome. That's why a differential diagnosis is crucial, so as to discern by which of them the patient is affected. The diagnosis is fundamentally made by recognition of their age of onset, the course and distinguishing clinical symptomatology associated, specific tests so as to know genetics, and molecule's measurements from muscle or skin biopsies (4). Despite the fact that an extreme display of genetic heterogeneity together with phenotypic similarities stand in the way of diagnosing, finding out an inheritance pattern is essential, as the majority of PMEs have autosomal recessive inheritance. Autosomal or mitochondrial inheritance is only been reported in rare cases (4,6).

4.2. LAFORA DISEASE (LD):

LD was first described in 1911, by Rodrigo Lafora and Glück. Lafora illustrated the hallmark of the disease in post-mortem studies: inclusions found in the brain of a myoclonic epileptic

patient, in 1911 (7). These aggregates were later found in other tissues apart from the brain and were named as Lafora Bodies (LBs).

But it was not until half a century later that a precise clinical description of LD was finally achieved in the Netherlands (8). Then in the following years, refinements have been added to the disease characterization.

LD conforms one of the progressive myoclonic epilepsies (PMEs) in adolescents. It is a neurodegenerative, rare and severe form of PME, with an autosomal recessive character. For that reason is quite common in pets and farm animals as a result of inbreeding. So in some cases animals as dogs (9) are used to study LD, and the comparisons between them and humans lead to surprising insights into genome biology (7).

LD classically appears in late childhood or early adolescence, without previous signs of sickness. Patients have been healthy before, but the fatality comes within a decade after onset, in most cases.

Only 20 years ago, the cause of LD was found to be the loss-of-function mutations in either EPM2A or EPM2B (NHLRC1) genes, which encode Laforin (*a glucan dual specificity phosphatase*) and Malin (an *E3 ubiquitin protein ligase 1*) respectively (3). Although the pathways followed by the enzymes is still under investigation, they regulate glycogen metabolism (10–12), and the result of its malfunction is a poorly branched and hyperphosphorylated glycogen that irremediably precipitates into polyglucosan aggregates known as *Lafora bodies* (LBs). This is the main reason that promotes the neurological degeneration development.

Laforin and Malin are an interacting complex. Normally, the first one recruits the other to glycogen chains while lengthening process, in order to counteract further extension. But the lack of one of them results in the branching inhibition and expulsion of water from the abnormal glycogen, leading to double helices formation and consequent precipitation and accumulation over time. This discovery placed LD in the context of glycogen metabolism disorders.

4.2.1. WHY IS IT SO UNKNOWN?

Some studies have settled LD's geographical distribution (13–15). Despite being a rare illness, the few reported cases around the world tend to gather at some focus (*see figure 1)*. Those include the Mediterranean region, especially Spain, Italy, France, and Northern Africa; Eastern Europe; and Asia, including southern India regions, and the Japanese and Chinese population.

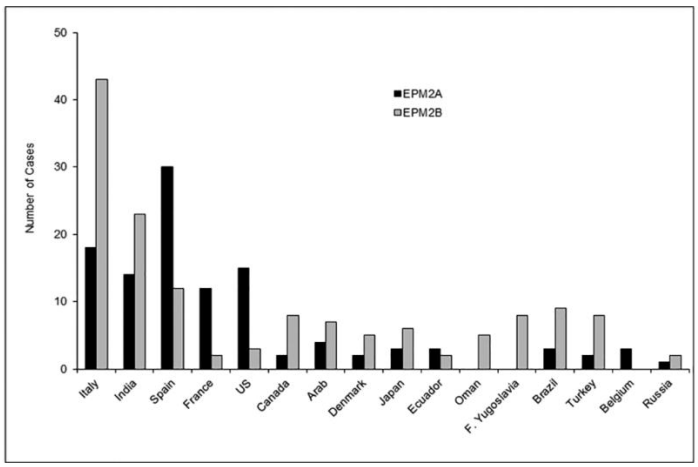


***Figure 1**: Number of LD cases (EPM2A and EPM2B mutation cases respectively) according to ethnicity or country, known by 2016, at the time the article was written* (3).

Because of its recessiveness, it's probable to find cases where consanguinity is high rated, although it can appear in any population because of DNA mutations that randomly occur.

The main reason why it is so unknown is because of its uncommonness. Epilepsy attacks are presented by approximately only 1% of the world population (16). From that percentage, just 1% of the epilepsies are PMEs (14), and then an even smaller portion corresponds to LD. Based on published reports of its causative mutations, it was estimated that the overall frequency of LD is of approximately 4 cases per million individuals in the world(3). Of these, 42% of the cases are caused by EPM2A mutations, and 58% by EPM2B. However, this ratio may vary in some populations.

Most PMEs are very rare, caused by isolated mutations in single families (7). Investigators and clinicians have limited experience with them, only the experts are aware of the regional most prevalent type of PME, which dominate thanks to local experience.

This lack of knowledge specially with the rare disorder LD, due to their uneven distribution around the world, must be solved somehow, as epilepsies are the most common neurologic disorder of children(14), and LD is a very incapacitating disease with no cure. So, although its low prevalence worldwide, its incidence within the affected families is high, and when a sibling gets diagnosed, any younger sibling may follow the same path. As families usually have several cases of LD, family burden, social and mental impact is very high. Even impact reflects on economic costs and obviously in the young patient's state of mind as everyday activities are limited (17).

4.2.2. NOWADAYS: ACTUAL TREATMENTS AND RESEARCH STRATEGIES

Current treatment strategies are based on the disease's progression, which has been classified for some studies (3,18) in the following clinical stages:

- Preclinical stage.
- Stage 1: In this stage some minor neurological impairments appear, but they do not affect daily activities yet. It is similar to juvenile myoclonic epilepsy (JME) and lasts from1 to 3 years.
- Stage 2: This stage is defined by a moderate neurological impairment, a disabling myoclonus and severe cognitive problems, so there is partial dependence for daily activities. It lasts from 2 to 5 years.
- Stage 3: This stage involves severe neurological impairment and total dependence for daily activities, so patients become wheelchair-bound or bedridden.

Patients require a clinical follow-up, psychological support and rehabilitation during all the disease, but according to the stages set before, they need specific care in each.

In early diagnosis of LD when the patient is in the preclinical stage, it is recommended to maintain schooling and social life as much as possible, combined with psychological support if needed. In stage 1, the previous recommendations are maintained, together with physical rehabilitation, while anticonvulsants therapy starts.

In the second stage LD is stablished, therapies set before should continue, and anticonvulsants may include a polytherapy. Also, home care adjustments or institutionalization might be required.

Finally, the last stage is characterized by full dependency and polytherapy failure. Treatment rests in prevention of status epilepticus. Hospitalization is needed for this critical care.

In general, for PMEs, treatment strategies combine antiepileptic and antimyoclonic drugs, including Valproate and Clonazepam respectively. The first one is used as the drug of choice, and the second one is used as add-on therapy (19,20), because it is the only drug approved by the FDA for myoclonic seizures.

Brivaracetam is still an antiepileptic experimental drug, but it has already been granted as orphan drug for symptomatic myoclonus treatment by FDA and EMA (21,22).

Contrarily, phenytoin should be avoided in all PMEs, due to its aggravating effect on neurologic symptoms and degeneration (23). Other drugs that also need to be avoided are sodium channel blockers, GABAergic drugs, gabapentin and pregabalin, as they may aggravate myoclonus (24).

When myoclonus aggravate, the avoidance of stimulus as light or noises is essential, as well as emergency treatments like intravenous benzodiazepines, valproate and levetiracetam, General anesthesia is rarely needed in exacerbations.

Finally, vagus nerve stimulating therapy has been proposed as it might reduce generalised seizures, but no conclusive results were obtained whether it was effective in outcome of myoclonic jerks and cerebellar function (25).

Although research in PMEs has advanced by leaps and bounds the last years, it is still in progress. With LD, the main goal is to find an effective treatment, as there is not any specific, but its research also opens a window into neuronal glycogen metabolism.

At present, treatment for LD remains palliative. The commonly used are antiepileptic drugs so as to improve symptoms in early stages(14,15), but even the best current therapies have limited success.

The lack of current understanding and effective treatments are driving investigations, including reduction in brain glycogen synthesis (by targeting GS or PP1 subunit R5), replacement of non-functional genes (by virus-mediated delivery of a functional Laforin or Malin gene), and even degradation of LBs by delivery of α-amylase has been proposed (26).

5. OBJECTIVES

Although it has been classified as a rare form of PME, LD cannot be ignored because of its low affectation rate. It is a devastating disease indeed, which has not only a huge repercussion on the patient, but also on the family and immediate surroundings as caregivers. Emotional, psychological and economic costs emerge from its fatality before adulthood.

Despite of recent progress made in understanding the genetics, neurobiology and physiopathology of the disease, there is still a long way to go, as nowadays there are only a few symptomatic drugs available that function temporarily just at onset. But, since neither specific nor effective treatment has been found yet, they are likely to prevail, unfortunately.

Due to LD's importance in our society, as one of the many diseases with moderate but unknown prevalence, the aims of this final degree project are:

- Review the pathophysiological basis of the PMEs, mainly LD.
- Describe LD's signs and symptoms.
- Describe LD's etiology, epidemiology and incidence.
- Study the molecular mechanisms implicated in LD.
- Review current diagnostic strategies for LD and suggest a modest personal contribution, suggesting an algorithm based on the symptomatology that might contribute to facilitate a diagnosis for doctors not specialized in this disease.
- Moreover, with an informational intention, design a paper leaflet/diptych to be published in order to make the disease known, or at least to try to increase awareness of rare diseases like LD, and promote both the investigation field and the support to patients and their families.

6. MATERIALS AND METHODS

This assignment is a bibliographic research. In order to achieve the objectives set before, an exhaustive and gradual research has been carried out.

In the first place, information about general epilepsy syndrome was recollected, in order to make a first approach and understand the basic aspects of this pathology. A physiology and physiopathology book (27), from the Faculty of Pharmacy's library was consulted. Some definitions, although, were found online in a medical dictionary.

In the second place, a bibliographic research was performed using two main platforms: PubMed and Scopus. Supported and developed by NCBI and NLM, and Elsevier respectively, they enable access to several databases such Medline, which is very important within medicine and health fields.

The majority of documentation obtained were from those online databases; search keywords were "Lafora" and "review", "Lafora" and "pathology", and "Lafora" and "diagnosis". Once general aspects of the disease were understood, more specific research was made with "Lafora", including "Laforin" or "Malin", "DNA mutations", "diagnostic methods" and "symptoms".

Bibliographic findings were mostly scientific articles and clinical reviews. Although great advances have been achieved in the past few years in the understanding of this disease, there are not sufficient studies yet. The range of dates is a really important criterion for research, but taking into account that this is a rare disease, which has little impact in the whole population, few studies have been performed and it is difficult to find new information. That's why some old articles were taken as valid for this final degree project.

Furthermore, some material was created (*see section 8. Elaborated material)* as a complement to this bibliographic research project:

On one hand, an interview to a health professional was developed so as to know an experienced point of view around clinical characteristics and treatment with patients, so it may contribute to a better understanding of the disease and its diagnosis. The questionnaire was carried out through google drive and it was sent to the department of genetic diagnosis from Vall d'Hebron Hospital and Sant Joan de Déu, both in Barcelona. Unfortunately, the attempt was unsuccessful, since no experienced doctor had met any case of LD.

On the other hand, an algorithm based on the differences between symptomatology of PMEs, and diagnostic methods available, has been suggested so as to be of help in diagnosis.

Finally, as set before in the objectives, this project aims to be informational and spread awareness about rare diseases as LD, so a paper diptych has been proposed. It includes the history and everyday life of an advanced fictitious patient. All with support of reliable information including signs, symptoms, and the cures and treatments that an advanced patient needs. The diptych also contains general information about LD, its repercussions, and the contact of several patient and family associations.

7. RESULTS AND DISCUSSION

7.1. LBs FORMATION AND DISTRIBUTION / PATHOGENESIS

The primary cause of LD is the deposition of polyglucosans (named LBs), due to non-functional mutations in EPM2A and EPM2B genes that cause the malfunction or the lack of Laforin-Malin interacting complex, responsible for breaking down abnormal glycogen.

a) LAFORIN AND MALIN COMPLEX

In one hand, EPM2A gene encodes Laforin, a protein with dual specificity phosphatase domain (DSPD), at the amino terminal, and a carbohydrate-binging domain (CBD), that binds to glycogen, at the carboxyl terminal (28,29). It has a cytoplasmatic localization, and it associates with the rough external surface of endoplasmic reticulum, binding to the ribosomes (30), and with the internal aspect of the plasmatic membrane (31). However, its localization is dynamic according to the cell's physiological state, for example, heat shock or glucose starvation induce its translocation to the nucleus (32).

In the other hand, EPM2B gene encodes Malin, an E3 ubiquitin ligase (33). It has cytoplasmic and nucleus localization (11), and its function is to ubiquinate proteins so as to promote their degradation, including Laforin (34) and other enzymes involved in glycogen metabolism, as glycogen-debranching enzyme (35), PTG/GS (36), AMPK (37), etc. Its self-interaction has also been reported, which may indicate that not only is involved in regulating Laforin's levels, but also its own levels, by auto-ubiquitination when its substrates are low (38).

b) LBs FORMATION

Glucan chains are made by chain-elongating (glycogen synthase) and branching enzymes (glycogen branching enzyme), that form α-1,4 and α-1,6 glycosidic linkages respectively. But glycogen is a heterogeneous mixture, with molecules with different lengths and consequently, different risks of precipitation. Too long and instable chains are modified by local reduction of chain elongation through several layers of quality control to avoid its deposition. Firstly, inhibiting locally glycogen synthase (GS), and secondly, with the removal of abnormal and precipitated glycogen. In the two layers of quality control, the Laforin-Malin complex play an essential role.

As a glycogen phosphatase, Laforin's function is to remove phosphates from glycogen's structure, allowing it to complete glycogen degradation by glicogen phosphorylase and glycogen debranching enzyme (39), so as to shorten chains that can remain soluble. Also, glycogen synthase kinase 3 (GSK3) is activated by Laforin's dephosphorylation, and GSK3 itself phosphorylates and inactivates glycogen synthase (GS) (40). So, a defect in Laforin could promote insufficient GSK3 activation and high GS activity (26).

Laforin binds preferentially to long glucan chains (26) that might be of risk. Malin, for its part, acts on Laforin in order to remove it from glycogen, so Malin is likely to be sequestered by longer chain molecules.

Therefore, Laforin and Malin are an interacting complex (36,41,42) with a critical role in glycogen metabolism, that helps to remain soluble in the cell as a large highly organised structure. When any of the two enzymes are missing, glycogen precipitates into a LB. They have a role in ubiquitin-proteasome pathway (43–45), autophagy (46,47) (as Malin has been reported to localize with processing cell bodies (48)), heat shock response (32,49), ER stress response (50), oxidative stress response (51,52), translational regulation (30), RNA metabolism (48), cell death pathway (53) and mitochondrial homeostasis (54).

The Laforin-Malin complex incorporate lysine 63 (K63)-linked polyubiquitin chains (37,55,56), which promote autophagic inclusions to degrade their Malin-mediated ubiquitynated targets (57,58). The complex is thought to downregulate glycogen chain elongation by targeting GS, and its activator, PP1 subunit R5 to degradation (26).

Another hypothesis (59) of LBs formation claims that the defection of Laforin induces phosphate accumulation, so glycogen becomes structurally abnormal and precipitates. Otherwise, the lack of Malin allows Laforin and other enzymes as GS (3) to remain joined to glycogen, disturbing its structure.

c) LBs DEFINITION AND DISTRIBUTION

LBs are considered pathogenic (4). They are depositions of glucose polymers, which consist of fibrillary polysaccharides that are poorly-branched, insoluble, highly phosphorylated, densely packed, and relatively resistant to amylase digestion (60), compared to glycogen. They are PAS-positive and vulnerable to acid hydrolysis, which ensures their glucose-based composition. Along with the filaments, other granular material lie within the LB (3).

LBs are comparable to other disease's polyglucosan bodies, as human corpora amylacea's structure and composition(14), but with differences in the age of appearance, localization and etiology. However, LBs are more similar to plant starch, as they are insoluble, with cluster-like distribution of branching points, unevenly distributed (26), due to an imbalance of elongation and branching reactions over glycogen, resulting in deposits of long polyglucosans with double-helix formation.

They are typically found in the brain, in the periportal hepatocytes of the liver, skeletal and cardiac myocytes and in the eccrine duct and apocrine myoepithelial cells of the sweat glands. They are scant in the cerebral cortex, but are numerous in the cortical neurophil (61–63).

Although glycogen is rarely found in neurons due to its rapid metabolization, LBs locate in the majority of neurons, with the highest proportion in the substantia nigra, followed by the dentate nucleus and thalamic nuclei (3). They specifically settle in cell bodies and dendrites (14,15), but not in axons, what might explain the cortical hiperexcitability typical of LD (3). Its mainly perikaryal distribution assimilates endoplasmic reticulum (14), as they are derived from it. That is why some LBs may present membrane in muscle tissue. This idea is supported by the absence of acid phosphatase and succinic dehydrogenase (60) in the vesicles, demonstrating that they are not lysosomes. However, they contain catalase and D-amino oxidase, suitable for peroxisomes (60).

7.2. SIGNS AND SYMPTOMS

Based on the published reports (3,14,64,65) classic LD consists of the following clinical manifestations:

7.2.1. PRESENTING SYMPTOMS

Before symptoms start emerging, patients have previous normal development during their first decade of life.

a) ONSET:

The beginning of the disease is considered to happen when any symptom manifests. This takes place between late childhood or early adolescence, which translates to the range of ages between 8 to 19 years. Specifically, the peak is located from 14 to 16 years, when LD is more likely to develop.

As mentioned before, LD has a high allele heterogeneity when talking about genotype. Many non-functional mutations appear in EPM2A and EPM2B/NHLRC1 genes, but with similar phenotype: the classical LD. There are only two exceptional forms of LD that present a different course:

In one hand, the early-onset LD (66), which presents itself early in childhood around the age of 6 years, as a result of a non-functional mutation in PRDM8 gene. In the other hand, there is a late-onset and slow progressive LD course (4,67,68), which is named *mild* LD, likely due to homozygous or heterozygous missense and non-functional mutations in NHLRC1 gene, from p.D146N chromosome.

As a consequence, when trying to recognize a case of LD by its age of onset, the rank of ages that should be considered is from 6 to 20 years.

Early characteristic manifestations are focal visual seizures (18). Other possibilities are generalized seizures and stimulus-sensitive myoclonus, which occur soon after.

In many cases, patients experience nearly simultaneous appearance of symptoms like headache, school difficulties, myoclonic jerks, generalized seizures and visual hallucinations (14,15,67).

Although brain MRI is unremarkable at onset, EEG abnormalities appear in this initial stage, often preceding clinical symptoms. It is characterized by general disorganisation with slowed posterior dominant rhythm, early disruption of sleep patterns and bursts of diffuse epileptic discharges.

By order of importance, the manifestations in early stages are as follows:

a) MYOCLONUS:

1% of epilepsies are PMEs, intractable myoclonic seizures joining progressive cortical degeneration that proceeds normal brain development in childhood (69).

Myoclonus are wreck contractions of a muscle itself or one part of it, or a group of muscles (1). It is characteristic of a convulsive disorder. It can present as fragmentary, arrhythmic, asymmetric or generalized seizures, and have a focal or segmental distribution.

It usually occurs at rest or by stimuli. Myoclonus aggravates with emotion, action (*intention myoclonus*) or photic stimulation. All kinds of myoclonus may appear either negative or positive, as momentary loss of tone or jerks respectively, but not all of them involve loss of consciousness.

As a result of photic stimulation myoclonus dependency, a marked photosensitivity manifests in LD patients.

In contrast to other PMEs, LD's myoclonus usually ceases with sleep, so no *nocturnal myoclonus* appears.

b) FOCAL VISUAL SEIZURES:

Partial visual seizures in LD appear as either transient blindness (14), which is a unique feature of LD in contrast to other PMEs, or visual hallucinations. Those hallucinations can be simple or complex (3,14), but they are not always due to epileptic cause, as some do not respond to antiepileptic treatment but do to antipsychotic (17,70).

The partial or focal seizures start in one side of the brain, these are occipital lobe photomyoclonic or photo- convulsions (14,64), what also contributes to photosensitivity.

Another manifestation associated can be classic migraine with scintillating scotomata(14,64).

c) IDIOPATIC GENERALIZED EPILEPSY:

In general, epilepsy is characterised by recurrent short attacks that imply motor, sensitive or physiologic dysfunction, without barely affecting intelligence in most cases. Known as "epileptic crisis", they are initiated by synchronized abnormal neuronal discharges at once, possibly due to wrong circuit excitability. As a result, muscular contractions can happen involuntarily, as well as visual or auditory signals might develop without previous stimulation of their pertinent organs (27).

Partial epileptic crisis have their origin in a delimited little focus on the brain, which cause a moderate form of the symptoms described before, whereas generalized epileptic crisis

encompasses bigger areas, including both sides of the brain, and with a conscious loss as a consequence. They can also be associated with fever in an early age appearance.

Many situations can be causative of epilepsy, as brain damage especially at birth, metabolic disruptions, infections, high level of toxins, vascular alterations, brain abscesses and neoplastic processes. For the most part, it is difficult to demonstrate a cause and LD is commonly missed.

Epileptic crisis can usually be treated effectively by administration of antiepileptic drugs as carbamazepin, fenitoin and valproic acid, among others. Even surgery would be an option for some drug-resistant diseases. However, LD's epilepsy is progressive and refractory to treatment.

In LD tonic-clonic seizures (14), absence, drop or atonic attacks, and complex partial seizures take place (71). Tonic-clonic seizures are characterized by muscle rigidity, forceful rhythmic contractions and loss of consciousness, with its usually abnormal EEG profile. Otherwise, absences attacks of diminished consciousness appear occasionally together with spasms and EEG showing a spike waves pattern (1).

d) SCHOOL DIFFICULTIES

Gradually deteriorating school results are a consequence of cognitive skills decline (71). It starts at soon or after clinical onset of LD.

e) EXCEPTIONS:

Although LD is not limited to the nervous system and LBs are present in other tissues, complications in other organs as cardiac dysfunction are rare (72), and hepatic insufficiency is an early event in rare LD patients (73).

7.2.2. CLINICAL COURSE

As LD progresses, the remaining asymmetric and segmental Myoclonus becomes almost constant and further massive myoclonic jerks appear.

Although in the first stage of LD the antiepileptic drugs might work, this increasing occurrence of seizures goes along with an increment of resistance to treatment. Therefore, any seizure type can develop to status epilepticus, which will be progressively more common.

Together with this progressively worsening condition of myoclonic epilepsy, other neuropsychiatric symptoms appear as behavioural changes, depression and apathy. According to the time succession, they are as follows:

a) EARLY APPEARANCE:

As a result of central or peripheral damage on nervous system, there appear lack of muscular control that results imperfect articulation of speech, known as dysarthria. This fact, accompanied by the manifestations set before, creates a general state of confusion in the young patient.

Furthermore, emotional disturbances as ataxia appear, which is also due to the lack of coordination of muscular activity during voluntary movement, resulting from a cerebellar or spinal cord disease's degeneration. Consequently, ataxia makes walking become gradually impossible.

b) LATE APPEARANCE:

In advanced stages, brain MRI might detect changes in the cerebral cortex, cerebellum and basal ganglia(18,74).

The damage in these structures promote aphasia, which characterises of partial or total loss of the ability to communicate, neither verbal nor written. It starts with difficulties in speaking, reading or writing, and continues with complications in recognizing objects or its names, understanding a conversation, etc. In addition, during some years the patient tries with efforts to maintain normal contact, with interrupted communication by extremely frequent myoclonic absence seizures, but in the end, speech becomes extremely difficult as a gradual and rapidly progressing dementia (71) appear. What is more, spasticity, even visual loss might develop (18).

Finally, as a result of mainly myoclonus mainly, apraxia (18) appears as the capacity to perform daily activities is completely lost by myoclonus and neurodegeneration, so that patients become wholly incapacitated and wheelchair bound, then, bedridden.

7.2.3. PROGNOSIS AND EVOLUTION OF PHENOTYPIC HETERO- AND HOMOGENEITY

The forecast of LD is still invariably progressive and lethal. As mentioned previously, genotype-phenotype correlation do not reveal considerable differences between patients carrying mutations in either EPM2A or EPM2B genes.

By definition, LD is heterogeneous genetically, but homogeneous phenotypically. However, there are always exceptions between that overall clinical uniformity. The appearance of symptoms and its succession are invariable, only timing and severity can vary.

Weather classic LD usually results from exon 4 mutations in EPM2A, which signifies only some of the functionality of Laforin is preserved, other few specific mutations appear to correlate with particular development of the disease.

In one hand, atypical early-onset variants of LD include two possibilities. Firstly, exon 1 mutations in EPM2A (75,76) imply a complete loss of Laforin's function, that leads to early-onset childhood learning disabilities, unlike classic LD. Secondly, mutations in PRDM8 gene, which is located in chromosome 4q21, brings with it a premature onset with isolated febrile or non-febrile seizures, myoclonus, ataxia and dysarthria.

In the other hand, atypical milder LD is a Malin-mediated variant, different from the previous. Its causes area homozygous or heterozygous missense and non-functional mutations in EPM2B/NHLRC1 gene, from p.D146N chromosome. Some of the Malin's functions are preserved, like the E3 ubiquitin ligase, but the capacity to interact with Laforin is weak (36). The result is not only a late onset, but a slow progressive course, with an extended preservation of daily living activities (11,77,78). However, this does not exclude a devastating disease course, it will come to a fatal end as Laforin-mediated LD.

From the same ancestral mutation, or between cases with allelic homogeneity, even among families, they might present clinical differences. It is thought that both genetic and environmental modifiers might influence this phenotypic spectrum (3,79).

7.2.4. FATAL CONSEQUENCES

Although LDs remarkable clinical and EEG features might lead to early diagnosis, due to the low incidence, few clinicians gain enough experience in this disease and therefore diagnosis is usually delayed.

Dealing with LD patients and families is not easy, as there is no hope to offer. Patients usually die within the following 10 years after clinical onset (3,14,64,65), succumbing to their illness' complications normally related to the degeneration of the nervous system and the refractory status epilepticus itself. They are completely bedridden in a nearly vegetative state as a result of cerebellar ataxia and dysarthria or mutism; but they might present psychosis states (17). Death finally comes often due to respiratory failure (14,15) or with aspiration pneumonia (3).

Some patients get intubated during their last days, under general anaesthesia, with continuous infusion of antiepileptic drugs as phenytoin (65).

Post-mortem brain biopsy studies show no demyelination, inflammation or deterioration present in neurons (14), although all regions of the CNS are involved to varying degrees in the neurological decline and significant symptomatology.

As inheritance is bilateral, from both parents, legitimate feelings of guilt must be mitigated. Moreover, as an important concern is the possible appearance of LD in other family members, it is explained to families that the risk can be excluded in older asymptomatic siblings, but not in younger or unborn siblings. Weather they have to be subjected to molecular screening is still in debate (3), as no effective treatment has been found to prevent or delay LD in pre-symptomatic cases. However, parents, siblings and other family members might benefit from molecular screening when planning to have more children.

Availability and financial aspects of diagnosis and genetic counselling, and its regulations differ between countries (3), so clinicians find themselves forced to conform to local laws.

7.3. DIAGNOSTIC METHODS

A typical case of LD can be in one of this three typical situations:

First, an advanced case that has not been diagnosed because of a lack of local expertise. This presents as an adolescent with a wide history of seizures, especially myoclonic, that seem to worsen during the last years, sometimes simultaneously to mental decline.

Second, an adolescent initially wrongly diagnosed as JME or IGE or photosensitive epilepsy, whose condition worsens and does not respond to antiepileptic treatment.

Third, a situation within the context of a family already diagnosed with LD in one of its members, where younger asymptomatic siblings may still manifest. In this case, diagnosis is essential to try a preventive treatment if necessary.

Onset of LD takes place between late childhood and early adolescence, before that, children appear to be completely healthy.

Initial symptoms that makes parents refer their child to the doctor may include myoclonus, for example involving a leg, and decrease in cognitive function, that manifests itself in increasing school difficulties. Indeed, during the anamnesis, the family usually can relate about elder family members having similar manifestations and expiring in a short period of time afterwards, which is obviously a significant concern for them.

During clinical examination and tests at onset, organs as heart, liver and kidneys appear to be within normal functional limits, and serological tests as HIV or hepatitis are negative. Even brain still has not any anatomical or pathological abnormality. Contrarily, EEG already show alterations in a characteristic pattern (65).

There are three levels of evidence that can lead to a suspicious case of LD:

1. Clinical evidence: including age of onset and PME's representative symptoms.
2. Complementary evidence: distinctive EEC and presence of LBs in skin biopsy.
3. Confirmation: Presence of non-functional homozygous mutations in one of the EPM2 genes, with heterozygous parents.

7.3.1. DIFFERENTIAL DIAGNOSIS BASED ON TYPES OF PMEs

One manner of starting to approach a diagnosis of LD is by dismissing other possibilities with exclusion criteria, based on the symptomatology presented and the age of onset, the circumstances and the progression of seizures.

Together with the difficulties described before, as there is an overlap of symptoms between PMEs and other epileptic and neurodegenerative diseases, the diagnosis of LD is challenging. In that sense, although general physical examination shows no abnormalities, clinical story is very important for the doctor, particularly the family background.

Besides, mental state or cognitive examination has to be evaluated to judge cognitive decline, including: alertness, orientation, mood, general behaviour, attention, recent and remote memory, serial subtraction, abstract reasoning, language reading, writing and reflexes (14,64). While in LD an initial normal neurologic examination might be possible, it rapidly unfolds dysarthria, ataxia, increased hallucinations, agitation and dementia (14).

Moreover, ophthalmologic examination and electroretinography are useful to distinguish LD from NCL and Sialidosis. Even cerebrospinal fluid levels of lactate and measles antibodies can dismiss MERRF and subacute sclerosing panencephalitis respectively (14).

In the Annex, there is a summary with the main characteristics of the most common PMEs in order to differentiate them from LD in the process of differential diagnosis. These criteria have been used to conform the diagnostic algorythm (*see section 8. Elaborated material).*

7.3.2. NEUROPHYSIOLOGIC STUDIES

Despite not being a specific diagnostic test of LD, **EEG** is helpful because its abnormalities precede clinical symptoms and might be useful for early detection of the disease (14).
Its characteristic disorganization (*see figure 2)*, with slow background activity, is suggestive of generalized epilepsy with encephalopathy, and it is combined with multifocal spike-waves discharges from right central and frontal region (64,65), as described on the table. This spike-waves pattern that triggered myoclonic jerks could be provoked by photic stimulation, which has to be also tested.

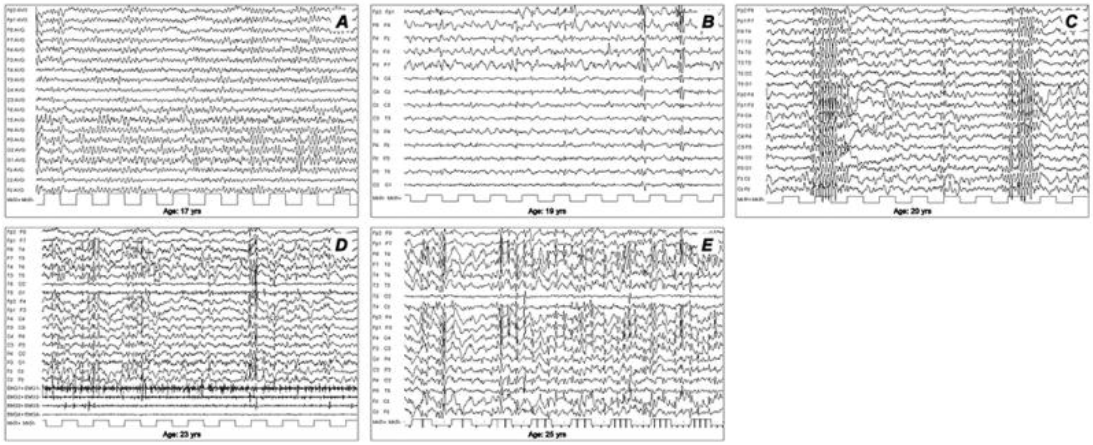


Figure 2*: Progression of electroencephalographic (EEG) changes in a patient with LD. Modified version of* (3) *from* (15).
(A) At onset (17 years-old), normal to slightly slowed background activity.
(B) Two years later, asymmetric generalized spikes and polyspikes, on a slowed background.
(C) At the age of 20, fast spike waves coexist with head drops.
During the final stages of LD EEG shows:
(D) Diffuse spike and polyspikes, associated with higher discharges or massive myoclonic jerks.
(E) Exaggerated worsening under photic stimulation.

Both mental state examination and EEG need to be repeated as time goes on so as to see the progression and neurodegeneration, if any.

Progression of the disease slows down EEG's background, even alpha-rhythm and sleep features fade, while photo-myoclonus increases (14,69). This last one is proceeded by a rapid spread of electrical transients going from the nape to the contralateral sensorimotor cortex, passing through ipsilateral *rolandic* area (80,81).

While in early years of LD, high-voltage somatosensory and visual-evoked potentials are present, with the progression of the disease, amplitudes may return to normal size, brainstem and central latencies extend, and also some auditory brainstem responses appear (14,69).

In addition, those initial occipital spike waves evolve to generalized spike-waves discharges, which increase irregularly filling the EEG with occipital predominance and focal abnormalities (14,69).

Otherwise, MRI volumetry of the brain present normal values for LD patients as compared to healthy controls, and no correlation between the disease's evolution and MRI measured volume change has been observed (18). But atrophy can appear with disease's advance (14).

Indeed, proton MR spectroscopy data show considerable metabolic changes especially in frontal and occipital cortex, basal ganglia and cerebellum, which are the most affected structures in LD. Statistically significant differences were found in the NAA/creatine ratio, which was reduced in LD compared to controls (18). In addition, NAA/choline and choline/creatine ratios also were statistically different only in frontal cortex (18). This is a sophisticated neuroimaging technique, which is not required for diagnosis, but these discoveries are remarkable for further studies of LD as a pattern of metabolic changes in the brain could be observed. Moreover, its distribution of the cerebral areas affected differ from that found in other forms of PME (18).

The predominance of intraneuronal LBs in structures as substantia nigra, thalamus, dentate nucleus and cerebral cortex has been associated with neuronal loss (62), however the relation between LBs and those metabolic changes revealed by spectroscopy is still unknown (18). However, neurotoxicity and stress due to a decreased ubiquitination system and reactive oxygen species (ROS) generation has been proposed (82).

In any case, the clinical suspicion set before with the differential diagnosis and neurophysiologic studies must be confirmed by the identification of LBs, which have been proven as pathologic (4), and the determination of mutations in EPM2A or EPM2B genes.

As observed in the two sections ahead, a great dominance of the clinical and pathologic peculiarities of PMEs is required to achieve rapid diagnosis, even not many pediatric neurologists know about LD, as it is a rare disease. For that reason, this project aims to make a modest contribution by an algorithm that may support the decision in differential diagnosis (*see section 8. Elaborated material).*

7.3.3. SKIN BIOPSY

LBs appear as cytoplasmic spherical to oval (64) polyglucosan inclusions, mainly spherical in neuronal tissue, but with an oval shape in non-neural cells as liver, muscle and in the epithelial cells (14), all ranging in size from 3 to 40 micrometers.

Despite LBs can be found in many tissues, skeletal muscle is not an ideal source for morphological diagnosis, since not all muscle fibre types are involved in this alteration. In addition, large portions of PASD positive material can be found in the periportal hepatocytes, as the hepatic pathology is not specific to LD alone. From the opposite position, skin enables a high sensitivity and less invasive test, as LBs can be found in the basal side of the apocrine acini *(see figure 3)* with a noticeable predominance in the myoepithelial cells, rather than secretory cells (almost 5:1) (83,84). Consequently, skin biopsy is of choice. It is taken from the axillary region, which is rich in sweat gland ducts, where LBs specially accumulate (84–86).

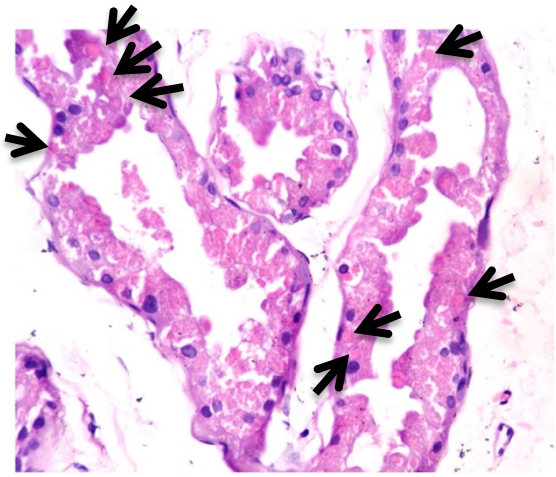


Figure 3: *Biopsy from axillary region shows intracytoplasmic spherical inclusions in the sweat gland duct (Haematoxylin and Eosin, 40X) (17).*

LBs locate close to the nucleus of either myoepithelial or secretory cells (*see figure 4)*, but often tend to saturate the entire cytoplasm as demonstrated by electron microscopy (64). Besides, they have not any limiting membrane in skin or brain cells, unlike in muscle and liver cells, where they do.

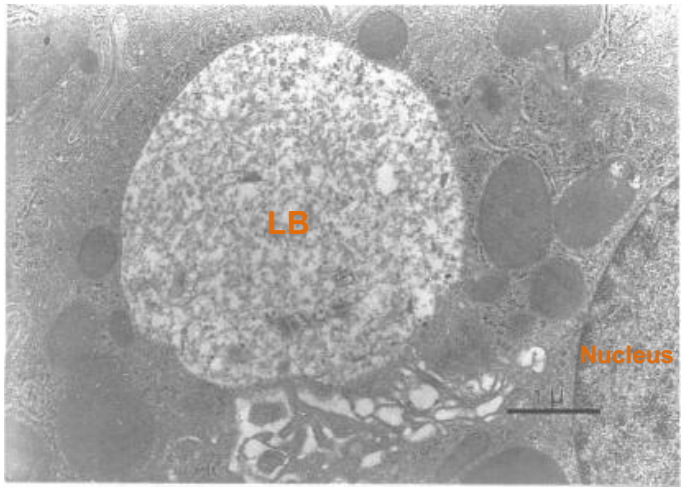


Figure 4*: Electron micrograph showing a secretory cell of an apocrine sweat gland containing a round inclusion body near the nucleus. It is composed of fine granular and filamentous material, but has no limiting membrane* (64).

In myoepithelial cells LBs locate usually in apocrine cells, but rarely in eccrine. However, the apocrine secretory cells never contain LBs, but do contain PASD-positive inclusions, which can be tricky (87). The only case in which a skin biopsy is misleading is in the LD variant due to PRDM8 gene mutations. In this case LBs are not present in sweat glands, so in a patient with positive clinical manifestations but negative skin biopsy, an open muscle biopsy should be considered (3,66).

LBs are diastase-resistant and stain strongly as acid-Schiff (PAS)-positive (65), which proves once more they have an important content of carbohydrates, appearing as fine granular and filamentous material (64) (*see figure 4 and 5)*. Furthermore, they show a dark densely-packed core and a paler diffuse peripheral zone (*see figure 6)*.

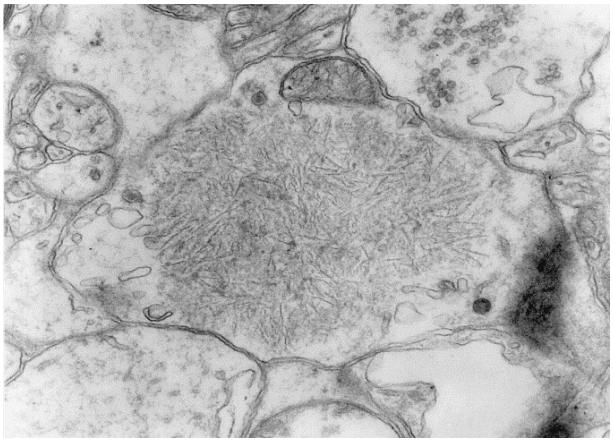


***Figure 5**:Electron micrograph of a Lafora body in a neuronal process (dendrite); polyglucosan fibrils can be appreciated. Original magnification × 30,000* (14).

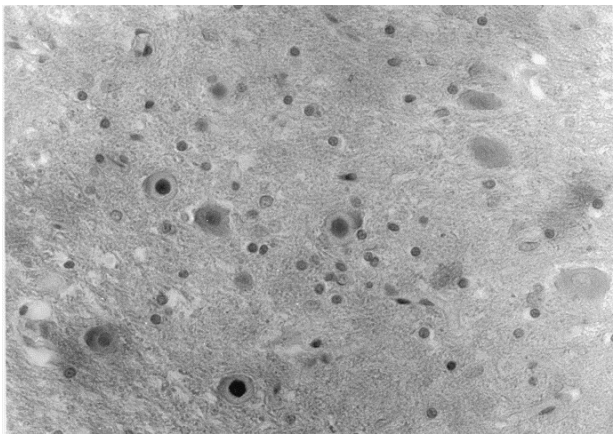


***Figure 6**: Numerous LBs with a dark core and a paler peripheral zone in dentate nucleus. Hematoxylin-eosin stain. Original magnification × 500* (14).

In addition, acid hydrolysis dissolves the inclusion, which supports the theory of its "polyglucosan" designation, as it reveals almost exclusively glucose molecules content (14).

LBs can be observed in optic microscopy, also with hematoxylin-eosin stain, as they are sufficiently distinctive. Keeping in mind that the PAS-reaction also reveals many small granules similarly composed, to distinguish this PAS-positive inclusions from the PAS-positive secretory material, a more intense lilac-purple staining is needed (83). Also it can be taken into account that LBs are larger than other dust-like particles stained PAS-positive, and in brain biopsy, those last concentrate in dendrites, while LBs are invariably perikaryal, like endoplasmic reticulum's distribution (14).

Moreover, an electron microscopy can be carried out in case that optic microscopy is unclear, but not when PAS-stained sections result negative already (64).

Apart from a skin biopsy, LBs can also be found in a brain biopsy, particularly in axons and astrocytes, and also in a biopsy of skeletal muscle and liver (88). Only in particular cases, however, a brain biopsy is used, usually post-mortem, when the diagnosis is not achieved on time and it is essential to define younger siblings' likelihood of developing similarly.

Taking into account that LBs are an undeniable attribute, even possibly the pathological source of LD, the detection of polyglucosan inclusions in cells are not exclusively for LD. Similar inclusions may be seen in other conditions as motor neuron disease, type IV glycogen storage disorder, arylsulfatase A pseudodeficiency, ayotrophic lateral sclerosis, and even in the process of normal aging (89), in which corpora amylacea are produced physiologically and can bear a likeness to LBs, despite not appearing in neuronal structures (64). Moreover, skin biopsy might not show the specific histomorphology (65).

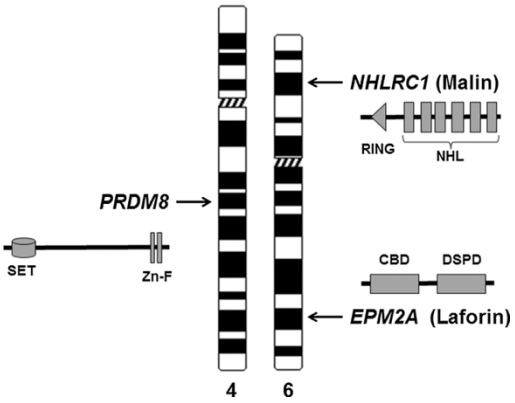
So, weather isolated LBs presence in sweat glands is the gold standard in LD's diagnosis, definitely required to reach a verdict, they are not a diagnostic feature alone. On top, this least invasive pathologic method of diagnosis has a very high (63,83), but not perfect sensitivity (90). So, to guarantee a solid diagnosis, another test like genetic mutation finding is needed. Only when genetic testing is not available or entirely conclusive, skin biopsy will be a diagnostic tool, but then a highly trained pathologist is required.

However, LBs are not found either in healthy subjects, or in other forms of myoclonus epilepsy (14,64). Thus, skin biopsy is a reliable test to approach and achieve early LD determination in an appropriate clinical context. The biopsy has been proposed to be performed even in patients with only myoclonus seizures, but without any other neurological symptoms yet (64), as there are mild forms of LD that progress slowly that could be detected in their early stages. It may also contribute to classify PMEs when the hereditary pattern remains unclear (64).

What is not yet proved is the early detection of LBs in axillary skin biopsy for siblings before the appearance of symptoms (64).

7.3.4. GENETIC TESTING

The first genetic locus discovered, whose mutations are causative for LD, was mapped on chromosome 6q24 (91,92). Then the responsible gene EPM2A was identified in 1998 (10,14,93), which afterwards was sequenced, finding out that it has 4 exons and codes for the protein named Laforin, a dual-specificity protein phosphatase (30) (*see figure 7*).



Years later, subsequent studies demonstrated a new locus of mutations mapping 6p23 chromosome, the EPM2B gene, also named NHLRC1 (11,12) (*see figure 7*). It is a single exon gene that codes for the protein Malin, an ubiquitin ligase (34).

***Figure 7**: Schematic diagram of the position of LD genes EPM2A and NHLRC1 on chromosome 6 and PRDM8 gene on the chromosome 4. The domain organization of the encoded proteins also appear (not drawn to scale)* (94).

Each of them account for about 50% of the patients, although it is believed that a third locus may exist, or a multiple genetic loci for LD might be possible (94), since some affected families don't have a mutation in either of the two genes described (95), but in PRDM8 gene. It was first described from a Pakistani family, located in chromosome 4q21 (66) (*see figure 7*), although its role in LD has not been established yet.

Only in the two genes EPM2A and NHLRC1 more than 150 distinct mutations have been found (3,13), including mutations like large and small deletions, insertions, point mutations, and mutations in the splice junctions (3,13,94). This extreme allelic heterogeneity in mutations, some of them extremely singular, even orphan, is an add-on challenge to diagnosis. Moreover, hemizygous deletions can be undetected in a conventional sequencing techniques as PCR (94), so it is critical to consider deletion/duplication analysis in suspected cases of LD when an initial sequencing of EPM2A and EPM2B reveal any variation (3).

Studies show that not all this mutation range variety is due to a founder effect, as a loss of genetic variation in some region, but to mutational hot spots with recurrent mutations in both genes (3,13), specific to particular ethnic groups and/or geographical regions. The most commons are p.R241X (in Spain) and p.G279S mutations to EPM2A; and p.P69A and p.G158fs16 mutations to NHLRC1 gene (75). Also, heterozygous mutations have been reported, suggesting the possibility of the existence of alterations in the other allele (11,12,75), contributing to the difficulties in genetic diagnosis set before.

LD is one of the recessive inherited PMEs (94), considered to be a clinically homogenous disorder, so it is difficult to distinguish which patients have loss-of-function and in which gene. Only a divergence in progression has been noticed, and this is that in general NHLRC1 defective patients present a slower disease progression (96), but some cases of EPM2A defects also show this slowness (97). Then, the variance in speed could be mutation specific (98), rather than gene specific.

Additionally, some subsyndromes with alterations in exons of the EPM2A gene have been reported, as carbohydrate binding domain (CBD), which is the first exon, and its malfunction leads to an atypical form of LD with childhood onset learning disabilities (75). Not only conventional mutations, but also the mutation on the alternatively spliced transcript of the EPM2A gene has been detected to possibly differ its effect (99).

Even a founder effect mutation in an Arabic family appeared to have variable expression, which may suggest the presence of modifier genes for those of LD (79). This hypothesis is reinforced by the fact that a study (100) reviewed that the same mutations in EPM2A showed variable clinical pathologies, and a variant sequence of protein targeting to glycogen (PTG) originated a slower progression of LD (100). In addition to that thesis, as Laforin and Malin form an interacting complex with many other proteins, then all those could be disease modifiers.

As mutations are uncountable yet, several variants of LD's clinical course have been reported, some as rare as late onset LD with parkinsonism (101), or even LD with obsessive compulsive symptoms (102).

In reference to genetic testing, although common mutations are rare, simple genetic tests have been set, as it is essential to establish which kind of PME is, in order to treat it before too much central nervous system (CNS) damage is done.

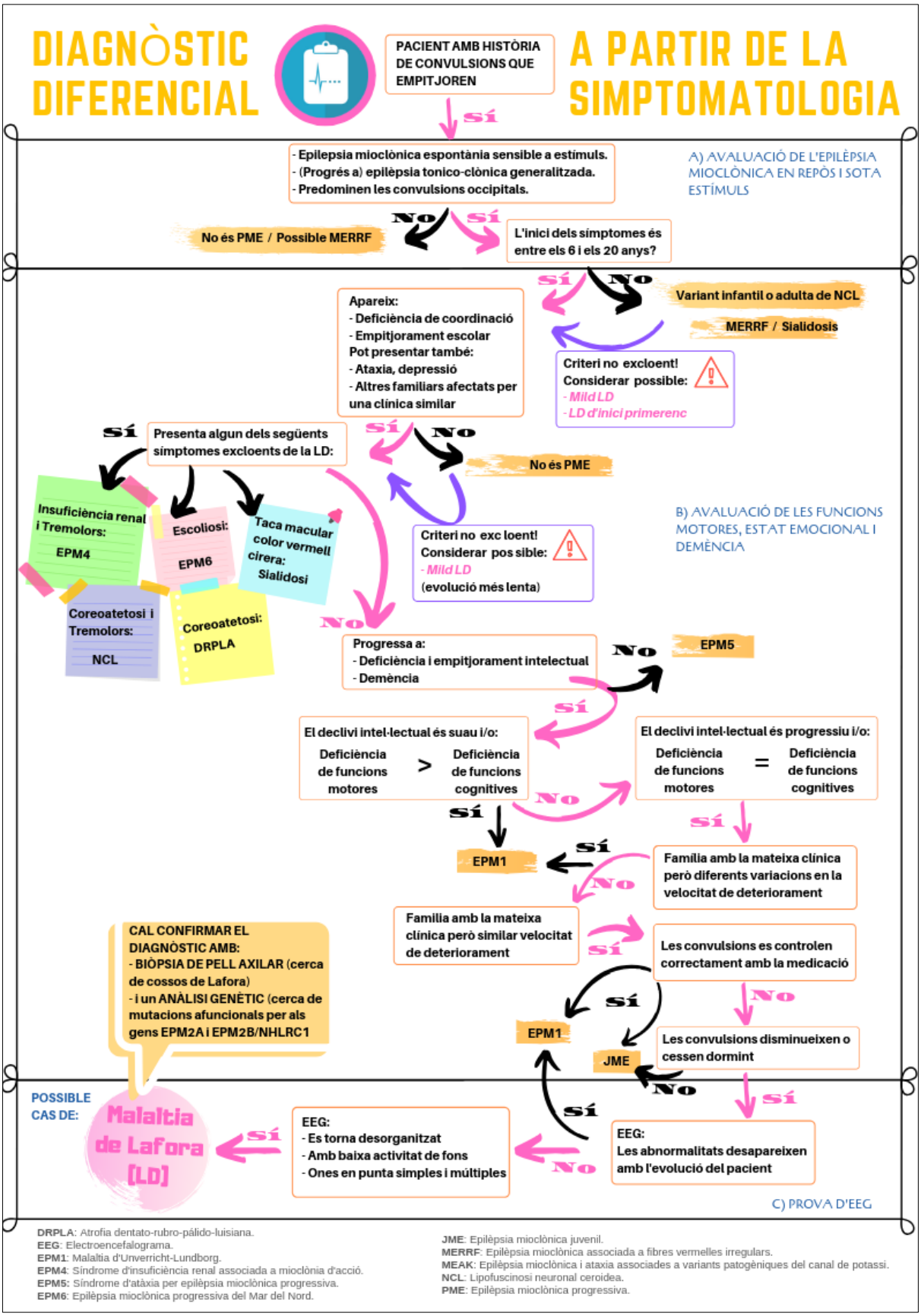
The gene defects causative of the most types of PMEs have been identified at present (4), but there does not appear to be any correlation between seizure type at onset and mutation type (71), which complicates their distinction.

Providentially, the two causative gens of LD have been shown to have a specific distribution of the mutations each. For example, EPM2A is more frequent in Spain, France and the US, weather NHLRC1 has a higher incidence in Italian, Arab, Indian, Canadian and Brazilian population (3,13), which may give a guideline when exploring gene defects.

The diagnosis will be confirmed when demonstrating a pathogenic mutation in both alleles of one of the EPM2 genes, together with the presence of heterozygous mutations in each of the clinically unaffected parents (3).

8. ELABORATED MATERIAL:

8.1. DIAGNOSTIC ALGORITHM:



8.2. INFORMATIVE DIPTYCH:

9. CONCLUSIONS

This research project helped me to accomplish the objectives set at the beginning.

- LD is a lethal PME caused by loss-of-function mutations generally in EPM2A or EPM2B/NHLRC1 genes, that encode Laforin and Malin respectively, but there is a wide range of pathogenic variants.
- Laforin and Malin interact as a complex. They take part in the glycogen quality control mechanism, so their dysfunctionality promotes abnormal glycogen structure, which precipitates into LBs, the unique cause of LD.
- Although genetic mutations are very heterogeneous, the phenotype is generally common. However, there are some atypical forms as early-onset or mild LD, with different time progression of the common symptomatology.
- Its low incidence and uneven world distribution make LD a unknown disease for most clinicians. Moreover, diagnosis of a specific form of PME is challenging, as they still remain uncommon disorders, and a certain level of expertise is required.
- Diagnosis is based on three levels of evidence. First, clinical features, including age onset and main symptoms as myoclonus and neurodegeneration. Second, complementary evidence as EEC and LBs' presence in skin biopsy. Finally, the last level is confirmation, which consists of genetical testing, in order to find non-functional mutations in the usual affected genes.
- EEC and skin biopsy are not just complementary evidence tests, they could be able to establish an early diagnosis, before clinical onset. Although prognosis is unfavourable, foreknowledge an early suspicion is essential to a rapid intervention to try to prolong life.
- Genetic counselling is essential as it provides information to families regarding mode of inheritance, natural course, treatment strategies available and what's more important, genetic risks to other younger family members. Furthermore, as a lifelong disease, patients and their surroundings have to cope with its consequences, therefore social and psychological support is needed.
- Most of the knowledge that we have about the neurodegenerative processes come from the learning path of new discoveries studying rare forms, as LD.
- Lack of current understanding and effective treatments drive further investigation, which is needed also to correlate genetic mutations with the clinical variation. Moreover, patients are scarce, which means there are no enough samples to investigate. In that sense, history shows that international collaboration and experience sharing is essential.
- The diagnosis of this disease means a devastating notice to the family, as losing one child is hard, but more than one is dreadful, and the idea of conceiving more children seem hopelessness, as they would be equally affected. Social, economic, physical and especially emotional impact is extreme for both the child and the family. In the last few years much progress has been made. Although the challenge is still huge, hope is set towards the investigation, which may find an effective treatment soon.

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11. ANNEX:

11.1 INTERVIEW

The interview to health professionals finally was unsuccessful, however, it is exposed as follows:

Entrevista: professional de la Salut - malaltia de Lafora

El meu nom és Paula Luque Gimeno (paula.luque@hotmail.com), sóc estudiant de farmàcia i estic fent el meu treball de fi de grau: Diagnosis and symptomatology of Lafora's disease.

La malaltia de Lafora (Lafora Disease, LD) és una malaltia rara neurodegenerativa que es troba dins el grup d'epilèpsies mioclòniques progressives i que es presenta inicialment en nens i joves des dels 6 als 20 anys. Actualment no hi ha diagnòstic previ a l'inici dels símptomes, a partir dels quals la malaltia es torna progressiva i refractària al tractament antiepilèptic. És autosòmica recessiva, i el 100% dels pacients tenen un final fatal aproximadament al cap de 10 anys de l'inici dels símptomes.

Aquest estudi té l'objectiu d'analitzar la base fisiopatològica de la malaltia de Lafora, incloent signes i símptomes, i descriure les estratègies diagnòstiques que es segueixen actualment. També té la intenció de fer una senzilla aportació personal suggerint un algoritme que podria contribuir a facilitar un diagnòstic per part de professionals no especialitzats en aquesta malaltia. A més, al relatar les característiques d'aquesta malaltia rara, amb un interès divulgatiu, s'espera que serveixi d'ajuda en donar-la a conèixer i, si més no, promoure el seu suport tant a la investigació com a l'atenció i suport dels pacients i les seves families.

En relació a aquesta intenció, es convida a participar a professionals de la salut en l'aport de coneixement sobre aquesta matèria. L'entrevista a especialistes en diagnòstic és essencial per rebre el seu punt de vista i explicació de les tècniques actuals, punts forts i febles. A més, els especialistes clínics poden oferir informació addicional sobre el tractament amb pacients, en la detecció dels símptomes, el tracte amb els afectats i el suport genètic.

La informació recollida s'utilitzarà únicament per finalitats acadèmiques, com a suport en aquest treball. Durant l'investigació només hi tindrà accés la investigadora responsable i la tutora en questió. Un cop finalitzat el projecte, passarà a formar part del dipòsit acadèmic de la UB on en cap cas s'utilitzaran per cap altre finalitat que no sigui acadèmica.

Moltes gràcies per la seva col·laboració.

Nom del participant i títol o càrrec professional:_____

La identificació del participant és important per donar validesa al estudi, la informació recollida s'utilitzarà únicament per finalitats acadèmiques. (exemple: Paula Luque - Farmacèutica del Hospital de...).

SÍMPTOMES I DIAGNÒSTIC CLÍNIC:

1. Quan es comença a sospitar d'un possible cas de LD?
2. Com s'identifiquen i/o diferencien les epilèpsies mioclòniques de les tonico-clòniques generalitzades? Es fa alguna mesura?
3. Quan arriba un possible cas de LD es fa un diagnòstic diferencial respecte les altres epilèpsies mioclòniques progressives (PMEs)?
4. La LD és una malaltia rara poc coneguda, hi ha gaire metges especialitzats en ella?
5. Disposen d'alguna guia o mètode estandaritzat per diagnosticar-la?
6. Ha tingut algun pacient amb LD? O un possible cas?
    a. Com se'n fa (o faria) el seguiment un cop diagnosticat? (proves, número de visites, seguiment farmacoterapèutic, etc…)
    b. Com va desenvolupar-se la clínica del cas que va tractar vostè? I les seves últimes fases?

DIAGNÒSTIC MOLECULAR I ASSESSORAMENT GENÈTIC:

7. Quan es sospita d'un cas de LD es fa una biòpsia de pell axilar (cerca de cossos de Lafora o inclusions de poliglucosan) i/o un anàlisi genètic (cerca de mutacions afuncionals dels gens EPM2A i EPM2B/NHLRC1), o una sola prova és decisiva?
    a. En cas negatiu, quin seria l'ordre de proves?
    b. Es fa alguna prova més?
8. En què consisteix una anàlisi genètica?
9. Quan es presenta un cas a la família, hi ha possibilitat que germans menors també presentin la malaltia.
    a. És posible fer un diagnòstic previ a l'inici dels símptomes?
    b. Es fa l'estudi genètic a la resta de familiars propers? O només als germans menors amb perill de presentar la malaltia també?
    c. Veu posible un cribatge prenatal en cas de dones embarassades?
10. En què consistiria l'assessorament genètic?

11.2. SUMMARY OF THE PMEs

Below there is a table with the main characteristics of the most common PMEs (4), in order to differentiate them from LD. This was of help when making the differential diagnostic (*see section 8. Elaborated material*).

PME	Aetiology	Epidemiology	Clinical symptoms	Further diagnostic investigations
EPM1	Is an autosomal recessive and progressive neurodegenerative disorder. It is caused by 14 known mutations in the cystatin B gene (CSTB), encoding a cysteine protease inhibitor that prevents intracellular degradation by lysosome leaked proteins.	It is a worldwide disease, with the highest incidence of all the PMEs. Mediterranean patients seem to present less severe clinical phenotype than Finnish ones.	Onset takes place between 6 and 16 years old. Early onset is related to a longer disease, more severe myoclonus and lower intellectual quotient. At start: - Involuntary action-activated or stimulus-sensitive myoclonus. It is asynchronized and progressive, and can occur in the proximal muscles of the extremities, being focal or multifocal, myoclonic jerks can develop to generalized status myoclonicus. - Generalized tonic-clonic epileptic seizures, which are usually well controlled with medication.	Should be considered at onset, when any symptom appears. Gene test: CSTB mutation analysis. The length of CSTB expansion mutation determines the age of onset, the myoclonus severity and cortical neurophysiology. Clinical examination: - Fundoscopy - Evaluation of motor functions. Its deterioration seem to vary within the family members. - Evaluation of emotional state. - Evaluation of myoclonus at rest and under stimuli by which could be detonated, including light, noise, stress and physical activity. - EEG: As a result of stimulus-sensitive myoclonus,

			As disease progresses: - Ataxia, depression, dysarthria, intention tremor and decreased coordination because of mild intellectual decline. The impairment is more significant in motor functions than in cognitive ones, such as memory and verbal abilities. - 33,3% of patients become wheelchair bound. Life expectancy nowadays is higher, approaching normal, but many years ago patients used to die before their thirties.	photosensitivity may occur, then generalized spike and polispike waves paroxysms during REM sleep, combined with background slowing on the EEG can be observed initially. But EEG abnormalities decrease as the disease stabilizes. - MRI at onset is typically normal. - Voxel-based morphometry may show cortical and thalamic atrophy, and thinner cortical sensorimotor areas.
LD (EPM2)	Is an autosomal recessive and progressive neurodegenerative disorder. It is caused by mutations in two known genes, EPM2A and EPM2B/NHLRC1,	Is a rare disease worldwide, but can be quite common in Mediterranean countries as Spain, Italy and France.	Symptoms onset occurs at late childhood or early adolescence. - General physical examination (from liver, spleen and heart) are always normal. - Elemental neurologic examination is usually normal at onset, but deteriorate presenting	Axillary skin biopsy to find Lafora bodies (LBs), acid-Schiff (PAS-) positive cytoplasmic inclusions distinctive of LD. They can be found in myoepithelial cells of the secretory acini of the apocrine sweat glands and in the eccrine and apocrine sweat duct cells. Gene tests in EPM2A or EPM2B/NHLR1 by mutation analysis.

	each one encoding proteins Laforin and Malin respectively.		dysarthia, spasticity and ataxia. - Mental state present visual hallucinations in epileptic attacks, depressed mood and cognitive deficiency as school difficulties from the beginning, and as LD progress, hallucinations increase, with the arrival of dementia, visual loss, apraxia and agitation. - Spontaneous and stimulus-sensitive myoclonus. - Generalized tonic-clonic or visual seizures. - 100% of patients become bedridden Excepting the begging of the course, both myoclonus and generalized tonic-clonic seizures are usually refractive to treatment.	- EEG presents disorganized at onset, with lightly slow background, multifocal, mainly posterior epileptiform discharges and generalized high-voltage spike and polispike waves. As disease progresses, the disorganization of EEG and photo-myoclonus do too, then epileptiform discharges become continuous. - Brain MRI scan does not show anatomical or pathological abnormalities, but might eventually show atrophy. - [^{1}H]MR Spectroscopy study show decreased NAA/creatine, NAA/choline and choline/creatine ratios as LD progresses. - Nerve conduction studies are normal along the LD. - CT scan is also normal. It can be confused with Juvenile myoclonic epilepsy (JME) at onset because of similarities. But, JME doesn't present neither slow

			Life expectancy: Death reaches all patients within a decade after onset.	background EEG nor neurologic deterioration with drug-resistant seizures. Age onset can be taken as an exclusion criteria, either start before 6 or after age 20 would not indicate LD. Moreover, further than 10 years evolution and absence of cognitive deterioration are also exclusive of LD. Slow progression is not an exclussion criteria, as there have been described slow progressive forms of LD, named as "**mild**". Similarly, there are forms of early-onset symptoms, which can be mistaken in exchange of infantile-variant neuronal ceroid lipofuscinosis.
NCL	Neurodegenerative diseases associated with lysosomal storage disorders. Caused by mutations on gens from CLN1 to CLN14.	common	It was used to be divided into infantile, juvenile and adult NCL subtypes, based on age of onset and symptoms presentation order. - Myoclonus and seizures, although they may be infrequent in some NCL forms. Myoclonus might	Skin biopsy to find granular osmophilic deposit. Leukocyte enzyme analyses: PPT1, TPP1, CTSD. Gene tests on 14 genetic forms (CLN1 to CLN14) mutation analysis.

			accentuate in late phases of progressive brain atrophy, together with involuntary movements and tremor. - Cognitive and motor decline - Retinal pathology and visual loss Life expectancy: Is related to the NCL type, but all lead to early death.	
Sialidosis	Autosomal recessive disease associated with lysosomal storage disorder. Caused by the deficiency of the gene NEU1 protein: α-N-acetyl-neuraminidase-1.		Different forms can present according to age onset and phenotype. - *Cherry-red spot myoclonus syndrome (Late and milder type 1):* exhibits myoclonic epilepsy, ataxia, visual worsening in patient's twenties or thirties. - *Infantile Sialidosis (type 2):* cognitive loss and myoclonus at the second decade of life.	Neuraminidase enzyme deficiency can be demonstrated in leukocyte enzyme analysis performed in cultured fibroblasts. Gen test on NEU1 mutation analysis. Clinical examination: - Macular cherry-red spot. - Sialo-oligosaccharides are found in urine, due to an increased sialic acid excretion.

			Life expectancy: Both lead to severe disability.	
MERRF	Mytochondrial syndrome associated with various mtDNA point mutations or POLG mutations.		Onset is variable. - Myoclonus, but it is not strictly linked to MERRF. - Generalized seizures, but less common in adults. - Ataxia	Plasma lactate and pyruvate. Presence of ragged-red fibers in muscle biopsy. Gene test: MT-TK mutation analysis.
Type 3 Neuro-nonpathic Gaucher Disease	Inherited disease associated with lysosomal storage disorder. Homozygous mutations result in deficiency of lysosomal glucocerebrosidase, that turns into accumulation of macrophages.		It can be classified in three types, the third with chronic and progressive neurodegeneration, myoclonus and PME.	Leukocyte enzyme analysis of ß-cerebroside. Gene test: GBA mutation analysis.
DRPLA	Autosomal dominant progressive disorder, caused by unstable		Age of onset and severity are determined by the repeat size of the mutation. Furthermore, the time of onset varies clinical presentation, including the	EEG background uses to be normal, with atypical spike waves. Gene test: DRPLA mutation analysis.

	expansion mutation in DRPLA gene, which codes for polyglutamine tracts.		following symptoms within the first two decades of life: - Myoclonus, epilepsy - Choreoathetosis - Ataxia - Cognitive decline and dementia - Photosensivity	
AMRF (EPM4)	Is caused by pathogenic mutations in SCARB2.		Onset is at the age of 15 to 25 years, either with proteinuria that progress to renal failure, or with neurologic symptoms such as: - Myoclonus, which leads to severe neurologic deterioration - Infrequent generalized seizures - Ataxia - Tremor Renal failure can be overcomed by renal transplantation, after surgery survival is feasible and cognition will be preserved.	Gene test: SCARB2/LIMP2 mutation analysis

PME-ataxia syndrome (EPM5)	Due to missense mutations in PRICKLE1 gene.		Onset is at 4 or 5 years, with presentation of ataxia and later myoclonus. Cognitive decline is mild or none.	Gene test: PRICKLE1 mutation analysis
North Sea PME (EPM6)	Caused by an homozygous mutation in GOSR2.	Its name reflects the proximity of the families affected to the North Europe, specifically, the North Sea.	Early onset presents at 2 years old, with ataxia, areflexia, elevated creatinine kinase values in serum, and myoclonus and epileptic seizures. As disease progresses, scoliosis appears at adolescence and finally patients loose independent ambulation, although cognition is preserved.	Gene test: GOSR2 mutation analysis
MEAK	Autosomal dominant inherited mutation.		Despite being more severe, onset parallels EPM1, starting between 6 to 15 years of age with myoclonus. As the disease progresses, myoclonus promote moderate incapacitation, and also appear infrequent tonic-colonic seizures, ataxia and mild or none cognitive decline.	Exome sequencing in patients previously subjected to negative molecular analyses.