

# Hepatic disease as the first manifestation of progressive myoclonus epilepsy of Lafora

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**ABSTRACT Background:** Lafora disease (LD; progressive myoclonus epilepsy type 2; EPM2) is an autosomal recessive disorder caused by mutations in the *EPM2A* and *EPM2B* genes. LD is characterized by the presence of strongly PAS-positive intracellular inclusions (Lafora bodies) in several tissues. Glycogen storage disease type IV (GSD-IV; Andersen disease) is an autosomal recessive disorder characterized by cirrhosis leading to severe liver failure. GSD-IV has been associated with mutations in the glycogen branching enzyme gene (*GBE*). Histopathologic changes of the liver in both diseases show an identical appearance, although cirrhosis has never been described in patients with LD. We report a LD family in which the proband presented severe liver failure at onset of the disease. **Methods:** Clinical histories, physical and neurologic examination, laboratory tests, EEGs, MRI of the brain, and liver or axillary skin biopsies were performed in the two affected siblings. The diagnosis was confirmed by molecular genetic analysis of the *EPM2A*, *EPM2B*, and *GBE* genes and loci. **Results:** During the first decade of life, abnormalities in liver function tests were detected in the two affected siblings. The proband's liver dysfunction was severe enough to require liver transplantation. Subsequently, both sibs developed LD. Mutation analysis of *EPM2A* revealed a homozygous Arg241stop mutation in both patients. **Conclusions:** This is the first description of severe hepatic dysfunction as the initial clinical manifestation of LD. The phenotypic differences between the two affected siblings suggest that modifier genes must condition clinical expression of the disease outside the CNS. **NEUROLOGY 2007;68:1369-1373**

Accumulation of polyglucosans represents the morphologic manifestation of several clinically heterogeneous disorders such as Lafora disease, glycogen storage disease type IV, adult polyglucosan body disease, and some cases of phosphofructokinase deficiency (Tauri disease).

Lafora disease or progressive myoclonus epilepsy of the Lafora type (EPM2 [MIN254780]) is an autosomal recessive form of progressive myoclonus epilepsy (PME) that initially manifests during adolescence and is characterized by epilepsy, myoclonus, and progressive neurologic deterioration. The diagnosis is sustained by the presence of periodic acid-Schiff (PAS) positive glycogen-like intracellular inclusion bodies (Lafora bodies). Lafora bodies, first described by Gonzalo R. Lafora in 1911, consist of an abnormal glucose polymer.<sup>1,2</sup> Lafora originally called the inclusion bodies "intracellular amyloid bodies" when he observed them in the brain and spinal cord of an adolescent patient who presented a progressive and fatal form of myoclonic epilepsy. In 1955, Harriman and Millar showed that the inclusions were not limited to the CNS and described similar intracellular inclusions in the heart and liver of one patient with Lafora disease.<sup>3</sup> Lafora disease initially manifests during adolescence, the most common age at onset being between 10 and 17 years. Generalized tonic-clonic seizures, absences, drop attacks, or partial visual seizures are usually the first manifestation, followed soon after by asymmetric as well as massive myoclonic jerks. As the disease progresses, the myoclonus increases in frequency and becomes constant. A rapidly progressive dementia

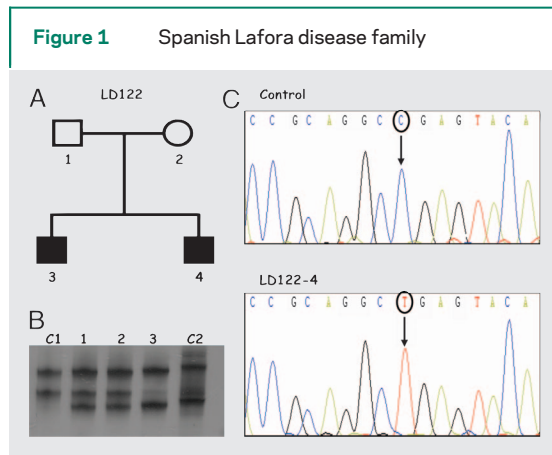
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(A) Spanish Lafora disease family. Black symbols indicate affected individuals. (B) Single-strand conformational polymorphism analysis. Individuals LD122-1, LD122-2, and LD122-3 are denoted as 1, 2, and 3. Individual C1 is an unaffected control. Individual C2 is affected with the Arg241stop mutation in homozygosis. Individuals 1 and 2 carry the mutation in heterozygosis and Individual 3 in homozygosis. (C) DNA sequence of exon 4 of the *EPM2A* gene showing the C→T change resulting in the replacement of arginine 241 by a stop codon.



with apraxia, aphasia, and visual loss ensues, leading patients to a vegetative stage and death, usually within less than a decade of first symptoms. Two genes have been associated with Lafora disease: *EPM2A* (chromosome 6q24) and *EPM2B* (chromosome 6p22.3). *EPM2A* encodes a protein tyrosine phosphatase (LAFPTPase or laforin).<sup>4,5</sup> In spite of the remarkable allelic heterogeneity in the *EPM2A* gene, the R241stop mutation has been found in approximately 40% of Lafora disease patients with mutations in this gene.<sup>6,7</sup> The *EPM2B* gene (also called NHLRC1) encodes an E3 ubiquitin ligase (malin) and the most frequent mutation found is P69A.<sup>8</sup>

Glycogen storage disease or glycogenosis type IV, also known as amylopectinosis or Andersen disease (MIN23250), is an autosomal recessive disorder caused by a deficiency of glycogen-branching enzyme (GBE) activity due to mutations in the *GBE1* gene.<sup>9</sup> A diagnosis can be made on the basis of the study of branching enzyme activity in erythrocytes.<sup>10</sup> Glycogenosis type IV is highly heterogeneous in terms of tissue involvement, clinical manifestations, and age at onset. The classic form of glycogenosis type IV is characterized by rapidly progressive hepatosplenomegaly and liver failure leading to either liver transplantation or death by the age of 5 years.<sup>9</sup> In addition to the classic form, variants such as a milder nonprogressive hepatic disease,<sup>11</sup> neuromuscular forms varying in onset and severity (from a fatal neonatal disease<sup>12</sup> to a mild adult myopathy<sup>13</sup>), a cardiopathic form of childhood,<sup>14</sup>

or a variant with multisystem involvement including liver and muscle<sup>15</sup> have been reported. GBE belongs to the alpha-amylase family and is required for glycogen synthesis. It catalyzes the formation of alpha 1,6-glucosidic branches that play an important role increasing the solubility of the molecule and is most highly expressed in liver and muscle. Typically, hepatocytes in glycogenosis type IV contain glycogen PAS-positive inclusions resulting in a ground glass appearance. This stored glycogen shows fewer branch points and longer outer chains resembling amylopectin. For this reason the disease is also referred to as amylopectinosis. Ultrastructurally, the inclusion bodies of Lafora disease and glycogenosis type IV appear quite similar.<sup>16</sup> Liver fibrosis, which can progress to cirrhosis, is a frequent finding in glycogenosis type IV. However, only mild to moderate periportal fibrosis has been described in the liver of patients with Lafora disease.<sup>17</sup>

Here, we present a clinical and molecular genetic study of two siblings with Lafora disease, one of whom developed a progressive liver cirrhosis and failure, requiring liver transplantation.

**METHODS Patients and samples.** We studied two siblings from a Spanish family (figure 1A). Both presented epilepsy, myoclonus, rapidly progressive neurologic deterioration, and a slow background activity with polyspike-wave complexes in the EEG.<sup>18,19</sup> A biopsy of skin from the proband and liver biopsy from the younger brother showed the characteristic PAS-positive Lafora bodies in both. Blood was collected from patients and their relatives after informed consent.

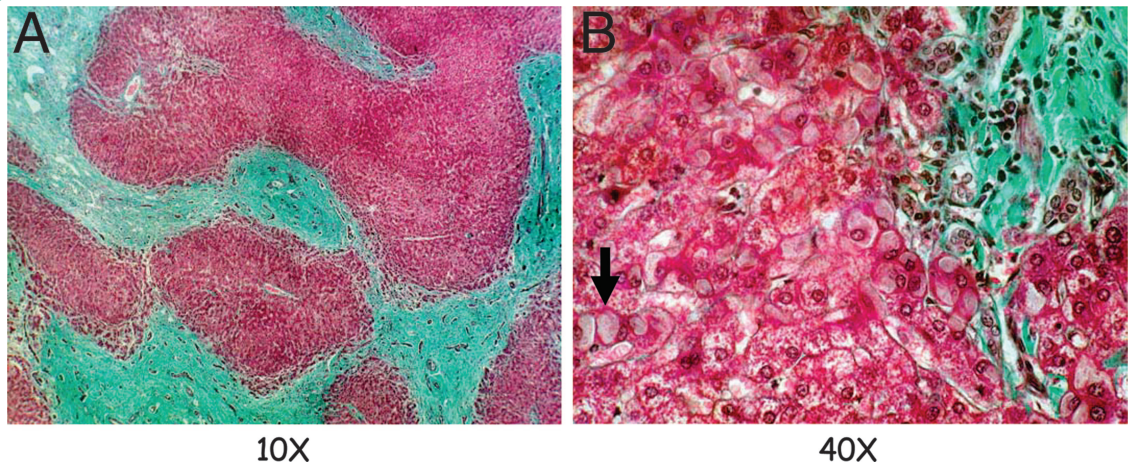
DNA samples were obtained from peripheral blood lymphocytes using standard methods. The study was approved by the Ethics Committee of the Fundación Jiménez Díaz.

**Microsatellite analysis.** Analysis of microsatellite polymorphisms on chromosomes 6q24 and 3p12 was performed by PCR using total human genomic DNA. Amplification was performed in a total volume of 10  $\mu$ L containing 40 ng of genomic DNA and isotopic phosphate. Samples were resolved on 6.5% polyacrylamide sequencing gels and bands were visualized using Kodak XAR films for 1 to 14 hours.

**Single-strand conformational polymorphism (SSCP) analysis.** SSCP analysis<sup>20</sup> was performed by PCR, using total genomic DNA, using the GenePhor DNA Electrophoresis System (Amersham Pharmacia Biotech). Amplification was performed in a total volume of 10  $\mu$ L containing 60 ng of genomic DNA. Samples were resolved on 12.5% non-denaturing polyacrylamide gels with the GeneGel Excel 12.5/24 kit (Amersham

(A) Histologic study of the liver of Individual LD112-3 showing loss of hepatic structure and confluent bands of collagen forming nodules (Masson's trichromic stain). (B) Ground-glass appearance of hepatocytes as expression of the Lafora bodies (arrow).

**Figure 2** Histologic study of the liver of Individual LD112-3



Pharmacia Biotech) and silver stained using the PlusOne DNA Silver Staining Kit (Amersham Pharmacia Biotech).

**Sequencing of exons.** Exons 1, 2, 3, and 4 of the *EPM2A* gene,<sup>6</sup> exon 1 of the *EPM2B* gene,<sup>8</sup> and exons 1 to 16 of the *GBE* gene were amplified from genomic DNA with specific primers using standard methods. The corresponding PCR products were purified by agarose gel electrophoresis and extracted with the Qiaquick Gel Extraction Kit (Qiagen). Direct sequencing of PCR products was performed with a dye-terminator cycle-sequencing kit (Perkin-Elmer) using *Taq* FS DNA polymerase. Sequences were resolved on an ABI PRISM 377 automatic sequencer, and the results analyzed with the ABI Analysis software (version 3.1).

**RESULTS Clinical findings. Case 1.** The proband presented growth retardation with height and weight deficiencies since early infancy. At age 7, laboratory tests revealed an increase in hepatic transaminase levels (alanine aminotransferase 321, aspartate aminotransferase 380) and abdominal distension, ascites, and increasing splenomegaly due to portal hypertension appeared. Hemorrhagic complications occurred subsequently and a severe hepatic failure was detected. A liver biopsy was performed at age 9. The histopathologic study showed ground-glass appearance of hepatocytes demonstrating positive Best carmine staining and lack of staining with orcein. Severe periportal stellate fibrosis was observed suggesting type IV glycogen storage disease (figure 2, A and B). He required liver transplantation at age 10. No clinical complications occurred during follow-up.

At age 16 he noticed transient light spots during a school test. He had his first generalized tonic-clonic seizure the following day. Six months later, myoclonic jerks were noticed. At first, these were asymmetric, segmental myoclonic jerks and occurred occasionally. During the following year, generalized tonic-clonic seizures increased in frequency and massive myoclonus appeared. Seizures were refrac-

tory to antiepileptic drugs. Progressive intellectual impairment occurred during the next 2 years.

An EEG showed diffuse slow background activity with bilateral paroxysms of spike-wave and polyspike-wave complexes. Marked photosensitivity was noted. Brain magnetic resonance showed cerebral atrophy and typical Lafora bodies were seen in an axillary skin biopsy.

**Case 2.** The proband's younger brother presented an increase of liver transaminases levels in a routine analysis at age 6 (alanine aminotransferase 112, aspartate aminotransferase 80). Although he remained clinically asymptomatic, a liver biopsy was performed at age 9. Liver biopsy revealed PAS-positive intracellular inclusion bodies with normal lobular architecture. No periportal fibrosis was observed. At age 14 he initially noticed occasional episodes consisting of seeing sudden and transient brilliant spots. These episodes lasted for few seconds and were especially frequent when he was watching television. Massive myoclonic jerks appeared 3 months later and he had his first generalized tonic-clonic seizure 1 year after the onset of neurologic symptoms. EEG showed a slow background activity, occipital spikes increasing with intermittent photic stimulation, and paroxysms of irregular spike-wave activity on both frontotemporal regions. During follow-up, myoclonic epilepsy and neurologic deterioration progressively developed but no hepatic dysfunction was detected.

**Genetic findings.** SSCP and mutational analysis of exons 1, 2, 3, and 4 of the *EPM2A* gene revealed a homozygous Arg241stop mutation in both siblings (figure 1, B and C), confirming the diagnosis of Lafora disease. Exons 1 to 16 of the *GBE* gene and exon 1 of *EPM2B* gene were also analyzed in both siblings in order to search for mutations. No mutations were found.

Haplotype analysis of the *EPM2A* and *GBE* genes was also performed and showed that both relatives presented identical haplotype for these loci (data not shown).

**DISCUSSION** Lafora disease has been considered a clinically homogeneous degenerative disorder manifested by progressive myoclonic epilepsy. Atypical Lafora disease with an early onset cognitive deficit phenotype has been recently reported by different authors in a minority of patients.<sup>21-23</sup> However, in no case of Lafora disease caused by mutations in *EPM2A* or *EPM2B* genes have initial clinical symptoms outside the CNS been reported. Here we describe the first Lafora disease family in which the proband presented a severe liver failure resembling glycogenosis type IV at onset of the disease. His younger brother showed abnormal liver function tests in a routine analysis although he was clinically asymptomatic and no signs of hepatic failure were detected during his follow-up. Both siblings subsequently developed the typical picture of progressive myoclonus epilepsy of Lafora type. PAS-positive inclusions were observed in an axillary skin biopsy from the proband and in a liver biopsy from his younger brother. The diagnosis of Lafora disease was confirmed by mutation analysis of the *EPM2A* gene, which revealed an Arg241stop mutation in homozygosis in both siblings. Interestingly, only the proband presented severe liver failure preceding the onset of progressive myoclonus epilepsy.

Histopathologic study of the liver in the proband showed ground-glass appearance of hepatocytes with PAS-positive inclusions and cirrhosis, leading to the diagnosis of glycogenosis type IV. Histopathologic changes of the liver in Lafora disease and glycogenosis type IV show an identical appearance. The similarity applies especially to the ground glass hepatocytes containing PAS-positive inclusions. Histochemical reactions of stored material are similar. However, patients with glycogenosis type IV typically develop cirrhosis while only mild to moderate periportal fibrosis has been described in patients with Lafora disease.<sup>17</sup> The clinical picture and the histopathologic findings in the proband strongly suggested the diagnosis of glycogenosis type IV. Mutation analysis of the complete coding region of the *GBE* gene did not reveal any mutations and haplotype analysis of the chromosomal region containing the *GBE* gene revealed identical haplotypes for both sibs, suggesting that this gene is not responsible for the liver failure present in the proband.

Similarities between Lafora and glycogenosis type IV diseases suggest that these two conditions

are closely related and may share a common metabolic disturbance conditioning failures at different levels of the same metabolic pathway. Concomitant enzyme deficiencies in the same individual causing an unusual glycogenosis with extensive neuronal polyglucosan storage has been reported.<sup>24</sup> This is an extremely rare condition but it is reasonable to think that a similar situation may account for the unusual clinical findings found in the patient here presented. This patient presents a combination of two different clinical phenotypes included in the group of glycogen metabolic pathway disorders. A homozygous mutation in *EPM2A* and no mutation in *GBE* were found. The most plausible hypothesis is that other unidentified modifier genes or genes involved in glycogen metabolism might condition the clinical expression of Lafora disease outside the CNS.

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