Lafora disease due to *EPM2B* mutations

A clinical and genetic study

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Abstract—*Objective:* To study *EPM2B* gene mutations and genotype-phenotype correlations in patients with Lafora disease. *Methods:* The authors performed a clinical and mutational analysis of 25 patients, from 23 families, diagnosed with Lafora disease who had not shown mutations in the *EPM2A* gene. *Results:* The authors identified 18 mutations in *EPM2B*, including 12 novel mutations: 4 nonsense mutations (R265X, C26X, W219X, and E67X), a 6-base pair (bp) microdeletion resulting in a two amino acid deletion (V294_K295del), a 4-bp insertion resulting in a frameshift mutation (S339fs12), and 6 missense mutations (D308A, I198N, C68Y, E67Q, P264H, and D233A). In our data set of 77 families with Lafora disease, 54 (70.1%) tested probands have mutations in *EPM2A*, 21 (27.3%) in *EPM2B*, and 2 (2.6%) have no mutations in either gene. The course of the disease was longer in patients with *EPM2B* mutations vs patients with *EPM2A* mutations. *Conclusions:* Genetic allelic heterogeneity is present in Lafora disease associated with mutations in *EPM2B*. Patients with mutations in *EPM2A* and *EPM2B* express similar clinical manifestation, although patients with *EPM2B*-associated Lafora disease seem to have a slightly milder clinical course. The lack of mutations in *EPM2A* and *EPM2B* in two families could be because of the presence of mutations in noncoding, nontested regions or the existence of an additional gene associated with Lafora disease.

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Lafora disease is characterized by epilepsy; fragmentary, segmental, and massive myoclonus; and rapid progressive mental deterioration.[1] EEG shows discharges of fast spike-wave and polyspike-wave complexes, photosensitivity, deterioration of background activity, and the appearance of multifocal abnormalities. Lafora disease is characterized by the presence of typical periodic acid-Schiff–positive (PAS) intracellular inclusion bodies (Lafora bodies)[2,3] composed of an abnormal glucose polymer that accumulate in the central and peripheral nervous system, among other tissues.[4,5]

Mutations in the *EPM2A* and *EPM2B* genes have been associated with Lafora disease. *EPM2A*, the major gene for Lafora disease, encodes a dual phosphatase known as laforin.[6,7] More recently, a second gene associated with Lafora disease, *EPM2B* (also called *NHLRC1*), was reported.[8] *EPM2B* codes for a putative E3 ubiquitin ligase,[8] known as malin. *EPM2B* is a single-exon gene spanning 1,188 base pair (bp) and codes for a 395 amino acid protein, containing a zinc finger of the Ring type and six NHL-repeat protein-protein interaction domains. The most common mutation, P69A, is located in the Ring finger domain.

Methods. *Clinical study.* We studied 25 patients, from 23 families, diagnosed with Lafora disease who had no mutations in the *EPM2A* gene. Patients originated from five Mediterranean countries (Spain, Turkey, Italy, Serbia-Montenegro, and France) and Ecuador. Diagnosis was based on the presence of epilepsy, myoclonus, rapidly progressive neurologic deterioration, and slow background with polyspike-wave complexes on EEG.[1,9] A skin, muscle, or liver biopsy also was required to confirm the presence of PAS-positive intracellular inclusions (Lafora bodies).[2,3,10] To compare patients with *EPM2B* mutations and patients with *EPM2A* mutations, we also studied 70 patients, belonging to 54 families, diagnosed with Lafora disease who had mutations in the *EPM2A* gene.

Molecular study. Blood samples were obtained after patients or their legal guardians signed an Ethics Committee-approved consent form. Genomic DNA was extracted from peripheral blood leukocytes using standard proceedings.[11] Linkage to the *EPM2A* gene locus (chromosome 6q24) was excluded by using microsatellite polymorphic markers D6S1703, D6S1649, and D6S1049 (data not shown).

Using four appropriate primer pairs,[8] the coding exon of *EPM2B* was amplified by PCR using 40 ng of genomic DNA. The corresponding PCR products were purified using the Montage PCR Centrifugal Filter Device Kit (Millipore, Billerica, MA) and directly sequenced with an ABI PRISM 3700 genetic analyzer (Applied Biosystems, Foster City, CA). Analysis was performed using the ABI Analysis software (version 3.1).

Mutations found by sequence analysis were tested by single-strand conformation analysis[12] in 100 control chromosomes using four additional pairs of primers (5'-ACCTCATAGAGCTCCTGGG-3', 5'-GGGCTCAGCGCTTCGCC-3', 5'-TTAGCTCAAGTATGCAGCTTG-3', and 5'-GTCGGCCAAGTGGATACCT-3') for PCR amplification (GenePhor DNA Electrophoresis System, Amersham Pharmacia Biotech, Uppsala, Sweden). Samples were re-

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solved on 12.5% nondenaturing polyacrylamide gels using the GeneGel Exel 12.5/24 kit (Amersham Pharmacia Biotech) and silver stained using the PlusOne DNA Silver Staining Kit (Amersham Pharmacia Biotech).

To associate haplotypes with mutations and to analyze the possibility of a founder effect or recurrent mutations, polymorphic markers spanning the *EPM2B* gene region were typed (D6S1721, D6S1653, D6S1605, D6S1567, D6S1688, D6S285, D6S507, and D6S1691).

Statistical analysis. SPSS software (Chicago, IL) for Windows (Microsoft, Redmond, WA) version 10.0 was used for statistical analysis. We used χ^2 analysis-of-contingency tables (with Bonferroni corrections when appropriate) to compare data from patients with *EPM2A* and *EPM2B* mutations. Patients were classified in four groups according to years of progression of the disease (from first symptoms to death): 1) <5 years; 2) 5 to 10 years; 3) 10 to 15 years; and 4) >15 years of progression. We also classified patients according to the age at which patients died in three groups: 1) age 10 to 19 years; 2) 20 to 29 years; and 3) >30 years. A patient who is still alive at age 37 years was included in the third group. Significance was assumed if $p < 0.05$.

The Ethics Committee of the Fundación Jimenez Díaz Hospital approved this study.

Results. *Molecular study.* Of 25 patients (23 families) with biopsy-confirmed Lafora disease and no mutations in *EPM2A*, mutations in *EPM2B* were found in 23. In two patients, no mutations were identified. Consanguinity was not present in the families of these two patients, and linkage analysis could not exclude linkage to the known loci because of their small size and poor informative characteristics. However, in one of these families, the proband and her 26-year-old unaffected sister had different haplotypes at the *EPM2A* and *EPM2B* loci.

We identified 12 novel mutations: 4 nonsense mutations (R265X, C26X, W219X, and E67X), a 6-bp microdeletion resulting in a two amino acid deletion (V294_K295del), a 4-bp insertion resulting in a frameshift mutation (S339fs12), and 6 missense mutations (D308A, I198N, C68Y, E67Q, P264H, and D233A). We also found six previously reported mutations.[8]

We also characterized the *EPM2B* haplotypes associated with each *EPM2B* mutation (table 1). P69A is the predominant mutation and was present in 14 chromosomes from nine individuals belonging to nine families. Comparison of haplotypes in patients with the P69A mutation showed that only Families 86 and 103 shared a common haplotype.

Clinical study. The clinical and neurophysiologic features of 17 affected individuals with mutations in *EPM2B* belonging to 15 families are summarized in table 2. No clinical or neurophysiologic information could be obtained from six patients. Consanguinity was present in five families (21.7%). Age at onset ranged from 12 to 15 years in all patients, excluding affected individuals from Families 127 (22 years) and 143 (7 years). Six different initial symptoms were identified: 1) a generalized tonic-clonic seizure was the most common initial manifestation and was the first manifestation of the disease in 50% of the patients; 2) simple partial occipital seizures (18.7%); 3) partial seizures with secondary generalization (12.4%); 4) absences (6.3%); 5) myoclonic seizures (6.3%); and 6) hepatic disease (6.3%).

For individuals with mutations in *EPM2A*, we identified seven different initial symptoms: 1) generalized tonic-clonic seizures (35.7%); 2) simple partial occipital seizures (17.8%); 3) partial seizures with secondary generalization (14.3%); 4) absences (14.3%); 5) myoclonic seizures (14.3%); 6) complex partial seizures (1.8%); and 7) hepatic disease (1.8%). Age at onset ranged from 8 to 17 years in all patients, excluding one affected individual with onset at 4 years. Age at which patients died ranged from 10 to 29 years, but most patients died between 19 and 29 years. In 65% of affected individuals, years of progression ranged from 5 to 10 years; in 25%, from 10 to 15 years; and in 10%, from 1 to 5 years.

Two illustrative cases of affected individuals with mutations in *EPM2B* are described below.

Patient 123-3. This patient had simple partial occipital seizures by age 12. The first myoclonic seizures occurred at age 13 to 14 years and were accompanied by generalized tonic-clonic seizures and absences. Photosensitivity was present. Inability to walk without assistance appeared at age 20 years. The patient died at age 35 years (years of progression, 23).

Patient 127-3. This patient had two isolated generalized tonic-clonic seizures at ages 5 and 6 years. The patient was in good health until age 22 years, when he had generalized tonic-clonic seizures. Since then, the neurologic picture has progressed slowly, with generalized tonic-clonic seizures, myoclonic seizures (onset, age 23 years), and cognitive decline (onset, age 28 years). At present, the patient shows resting and action myoclonus, mental deterioration, and cerebellar signs. Gait disturbance appeared at age 30 years. The EEG and skin biopsy were consistent with the diagnosis of Lafora disease. The patient is now age 37 years.

When we compared years of progression in patients with *EPM2A* and *EPM2B* mutations, we found that the course of the disease was longer in patients with mutations in *EPM2B* ($p = 0.011$ and 0.033 using Bonferroni correction). We also found that the age at which patients died was higher in patients with *EPM2B* mutations than in patients with *EPM2A* mutations. For patients with *EPM2A* mutations, the mean age at which patients died was 20.85 years, and for patients with *EPM2B* mutations, the mean age of death was 25.67 years. The difference was significant ($p = 0.014$ and 0.042 using Bonferroni correction). Two patients with mutations in *EPM2B* reached the fourth decade of life. No significant clinical differences were observed between patients with *EPM2A* and *EPM2B* mutations for other variables studied (age at onset, initial symptom, and photosensitivity).

Discussion. In this report, we describe a systematic mutational analysis of the *EPM2B* gene in 46 chromosomes from 23 patients with Lafora disease (21 unrelated). We have identified 18 *EPM2B* mutations, 12 of them novel. In our series of 95 patients, 70 (73.7%) patients with Lafora disease had mutations in *EPM2A* and 23 (24.2%) in *EPM2B*, and in 2 patients (2.1%), no mutations were found in either gene. With respect to families, 54 (70.1%) families had mutations in *EPM2A*, and 21 (27.3%) had mutations in *EPM2B*; in 2 (2.6%) families, no mutations were found in either gene. Our data suggest that *EPM2A* and *EPM2B* are the major genes for Lafora disease, representing >95% of the patients.

We have also characterized the *EPM2B* haplotypes associated with each *EPM2B* mutation. P69A is the predominant mutation and was found in 14 chromosomes. We compared P69A mutation haplo-

Table 1 EPM2B *mutations and haplotypes associated with* EPM2B *mutations*

Mutation	Nucleotide change	Type	Freq	D6S1721	D6S1653	D6S1605	D6S1567	D6S1688	D6S285	D6S507	D6S1691	Origin
C68Y	203G→A	Missense	2	255	154	121	114	172	213	212	215	France (55a)
				263	156	131	114	172	213	216	215	France (55b)
W219X	656G→A	Nonsense	2	255	—	137	118	184	215	198	—	Spain (63a)
				255	—	137	118	184	215	198	—	Spain (63b)
P69A	205C→G	Missense	14	257	154	135	108	176	215	216	223	France (86a)
				257	154	135	108	176	215	216	223	France (86b)
				263	154	135	108	176	215	—	223	Spain (103a)
				263	154	135	108	176	215	—	233	Spain (103b)
				257	156	—	116	—	215	204	215	Spain (123a)
				255	162	—	116	—	215	204	215	Spain (123b)
				265	156	131	114	176	207	228	223	Italy (138a)
				265	174	137	114	176	207	226	227	Italy (138b)
				253	162	127	108	176	213	216	219	Spain (146a)
				265	162	127	108	176	213	216	225	Spain (146b)
				257	152	129	116	170	223	226	213	Spain (120a)
				261	156	135	116	170	213	220	213	Spain (143b)
				265	176	129	110	172	213	—	227	Italy (148b)
				—	—	—	—	—	—	—	—	Italy (147)
V294_K295del	880–885delGTGAAA	Deletion	1	253	154	133	116	170	219	210	231	France (9577b)
E67Q	199G→C	Missense	1	255	154	131	118	170	213	220	225	France (9577a)
E67X	199G→T	Nonsense	2	255	154	125	118	180	217	220	221	Turkey (167a)
				259	154	133	118	180	217	220	223	Turkey (167b)
P264H	791C→G	Missense	2	255	172	141	108	168	207	212	227	Spain (104a)
				257	172	141	108	170	207	212	227	Spain (104b)
G158fs73	468delA	Deletion/ frameshift	1	255	152	133	116	176	215	216	219	Spain (120b)
D232A	698A→C	Missense	2	255	154	133	116	180	219	224	223	Italy (124a)
				255	178	133	118	180	219	224	229	Italy (124b)
S339fs12	1017–1018insATCT	Insertion/ frameshift	1	253	182	133	116	170	209	210	215	Italy (127b)
D146N	436G→A	Missense	1	255	154	133	118	170	215	214	229	Italy (127a)
E350fs46	1048–1049delGA	Deletion/ frameshift	2	251	154	135	108	172	213	210	213	S-M (137a)
				253	154	145	108	168	213	210	215	S-M (137b)
E280K	838G→A	Missense	2	265	162	135	110	176	215	—	223	Italy (148a)
				—	—	—	—	—	—	—	—	Italy (147)
R265X	793C→T	Nonsense	2	255	178	135	118	170	221	204	213	Turkey (175a)
				255	178	135	118	170	221	204	213	Turkey (175b)
C26X	78C→A	Nonsense	1	255	152	135	116	168	209	204	233	Spain (143a)
G158fs16	468–469delAG	Deletion/ frameshift	1	—	—	—	—	—	—	—	—	Italy (142)
I198N	593T→A	Missense	2	259	156	—	116	182	207	208	233	Ecuator (176a)
				269	160	—	116	182	207	208	231	Ecuator (176b)
D308A	923A→T	Missense	2	—	162	135	120	180	213	—	—	Italy (174a)
				—	154	135	120	180	213	—	—	Italy (174b)

Haplotypes from families 142 and 147 could not be characterized because of lack of DNA from parents.

S-M = Serbia-Montenegro.

type constructions. Families 86 (France) and 103 (Spain) shared a common haplotype between D6S1653 and D6S285, suggesting a founder effect. The P69A mutation in the other families may have different phylogenetic origins, implying recurrence of the mutation. All other mutations were found only in one or two chromosomes. Failure to detect the second *EPM2B* mutation in one family (142) does not exclude the presence of a second mutation because we did not test noncoding regions, such as the promoter region.

Table 2 *Clinical features and age at onset of 17 affected individuals with* EPM2B *associated Lafora disease*

	Mutation																
		P69A P69A			P264H P264H	G158fs73 P69A	S339fs12 D146V	E350fs40 E350fs40		C26X P69A		E280K P69A		E67X E67X	D308A D308A	R265X R265X	I198N I198N
Family	103	123	138	146	104	120	127	137–3	137–4	143	147–3	147–4	148	167	174	175	176
Country	Spain	Spain	Italy	Spain	Spain	Spain	Italy	Serbia/Montenegro		Spain	Italy	Italy	Italy	Turkey	Italy	Turkey	Ecuador
Age at onset, y	14	12	12		14–15	12	5/22	12	12	7	12	13	13	13,5	16	13	14,5
Initial Symptom	GTCS	SPOS	GTCS	HF	SPOS	PSSG	GTCS	A	SPOS	GTCS	GTCS	GTCS	GTCS	GTCS	MS+A	MS	PSSG
Age at onset of seizures and other symptoms and signs, y																	
SPOS	—	12	—	—	14–15	—	—	15	12	—	—	—	—	—	—	—	14,5
GTCS	14	13	12	—	16	12	5/22	14	12	7	12	13	13	13,5	—	—	14,5
CPS	—	—	—	—	—	13	—	—	—	—	—	—	—	—	—	—	—
A	—	13	—	—	14–15	12	—	12	—	—	—	—	—	—	16	—	14,5
Tonic	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Atonic	—	—	—	—	—	14	—	—	—	—	—	—	—	—	—	13	—
MS	15	13	12	—	16	13	23	16	13	14	13	13	14	14,5	16	13	14,5
Deterioration of cognitive function	15	—	15	—	17	14	28	15	15	14	16	16	16	14	18	—	12
Inability to attend school	—	—	16	—	—	—	—	16	15	—	16	16	16	14	—	—	12
Gait disturbance	—	—	16	—	17	—	30	16	16	—	16	17	17	—	—	—	—
Inability to walk alone	—	20	17	—	18	—	—	17	—	23	16	17	17	16	—	—	—
Complete deterioration of mental status	—	—	17	—	—	—	30	16–19	17	14	17	17–20	17	16	—	—	—
Age at which patients died, y	—	35	—	—	—	—	—	20	—	—	20	22	—	20	—	—	—
Years of progression	9	23	9	—	4	7	16	8	5	16	8	9	4	6,5	3	—	—

GTCS = generalized tonic-clonic seizure; SPOS = simple partial occipital seizure; HF = hepatic failure; CPS = complex partial seizure; PSSG = partial seizure, secondarily generalized; A = absences; MS = myoclonic seizure; — = not determined.

Lafora disease initially manifests during adolescence, the most common age at onset being between 10 and 17 years.[1] Patients die within less than a decade of first symptoms. Here we report 12 patients with *EPM2B* mutations with homogeneous clinical presentations and 5 additional patients with an atypical presentation (probands from Families 123, 127, 143, 146, and 176). The affected individual from Family 127 had a late onset (22 years) and is still alive at age 37 years. The affected patient from Family 123 had a long progression and died at age 35 years. It appears that in Lafora disease progression is longer if *EPM2B* is mutated. Statistical analysis confirmed this hypothesis.

An early-onset subphenotype consisting of cognitive deficit has been reported to be associated with mutations in the first exon of *EPM2A*.[13,14] Here we report a patient (proband from Family 176) with learning disorder, followed by epilepsy and neurologic deterioration and a homozygous mutation in *EPM2B*. The mutation in Family 176 is located in an NHL protein-protein interaction domain.[15] It may be that additional factors besides the mutated exon or gene are involved in early-onset cognitive deficit. Interacting properties of laforin[16,17] and malin may play an important role in this subphenotype.

Other families with atypical phenotypes are Families 143 and 146. The affected patient from Family 143 had an early age at onset (7 years) and has a long progression of 16 years (the patient is still alive). Both mutations of this patient are located in the Zn^{2+} Ring finger domain,[18] as in Family 123, but no clinical similarities were found between affected of both families. In Family 146, the initial symptom was hepatic failure, and to date no neurologic manifestations have appeared. The mutation in this patient is also located in the Zn^{2+} Ring finger domain.

The observed differences in the phenotypic expression between patients with *EPM2A* and *EPM2B* mutations could be because of the role of malin. Malin is a putative E3 ubiquitin ligase[8] involved in proteolysis cascades.[19,20] It may be that other E3 ubiquitin

ligases may reduce the effect of *EPM2B* mutations, increasing the years of progression and delaying the age at which patients die compared with those with *EPM2A* mutations.

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References

1. Van Heycop Ten Ham MW. Lafora disease, a form of progressive myoclonus epilepsy. In: Vinken PJ, Bruyn GW, eds. The epilepsies: Handbook of clinical neurology, vol 15. North-Holland, Amsterdam: Elsevier, 1975:382–422.
2. Lafora GR. Über das Vorkommen amyloider Körperchen im Innern der Ganglienzellen; zugleich ein zum Studium der amyloiden Substanz im Nervensystem. Virchows Arch [Pathol Anat] 1911;205:295–303.
3. Lafora GR, Glueck B. Beitrag zur Histopathologie der myoklonischen Epilepsie. Z. Gesamte Neurol Psychiatr 1911;6:1–14.
4. Yokoi S, Austin J, Witmer F, et al. Studies in myoclonus epilepsy (Lafora body forms). I. Isolation and preliminary characterization of Lafora bodies in two cases. Arch Neurol 1968;19:15–33.
5. Sakai M, Austin J, Witmer F, et al. Studies in myoclonus epilepsy (Lafora body form). II. Polyglucosans in the systemic deposits of myoclonus epilepsy and in corpora amylacea. Neurology 1970;20:160–176.
6. Serratosa JM, Gomez-Garre P, Gallardo ME, et al. A novel protein tyrosine phosphatase gene is mutated in progressive myoclonus epilepsy of the Lafora type (EPM2). Hum Mol Genet 1999;8:345–352.
7. Minassian BA, Lee JR, Herbrick JA, et al. Mutations in a gene coding a novel protein tyrosine phosphatase cause a progressive myoclonus epilepsy. Nat Genet 1998;20:171–174.
8. Chan EM, Young EJ, Ianzano L, et al. Mutations in NHLRC1 cause progressive myoclonus epilepsy. Nat Genet 2003;35:125–127.
9. Tassinari CA, Bureau-Paillas M, Dalla-Bernardina B, et al. La maladie de Lafora. Rev EEG Neurophysiol 1978;8:107–122.
10. Shirozu M, Hashimoto M, Tomimatsu M, Nakazawa Y, Anraku S, Nagata M. Lafora disease diagnosed by skin biopsy. Kurume Med J 1985; 32:311–313.
11. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning. A laboratory manual, 2nd ed. New York: Cold Spring Harbor Laboratory Press, 1989.
12. Orita M, Iwahana H, Kanazawa H, et al. Detection of polymorphism of human DNA by gel electrophoresis as single-strand conformation polymorphism. Proc Natl Acad Sci USA 1989;86:2766–2770.
13. Ganesh S, Delgado-Escueta AV, Suzuki T, et al. Genotype-phenotype correlations for EPM2A mutations in Lafora's progressive myoclonus epilepsy: exon 1 mutations associate with an early-onset cognitive deficit subphenotype. Hum Mol Genet 2002;11:1263–1271.
14. Annesi G, Sofia V, Gambardella A, et al. A novel exon 1 mutation in a patient with atypical lafora progressive myoclonus epilepsy seen as childhood-onset cognitive deficit. Epilepsia 2004;45:294–295.
15. Slack FJ, Ruvkun G. A novel repeat domain that is often associated with RING finger and B-box motifs. Trends Biochem Sci 1998;23:474–475.
16. Wang J, Stuckey JA, Wishart MJ, Dixon JE. A unique carbohydrate binding domain targets the lafora disease phosphatase to glycogen. J Biol Chem 2002;277:2377–2380.
17. Fernandez-Sanchez ME, Criado-Garcia O, Heath KE, et al. Laforin, the dual-phosphatase responsible for Lafora disease, interacts with R5 (PTG), a regulatory subunit of protein phosphatase-1 that enhances glycogen accumulation. Hum Mol Genet 2003;12:3161–3171.
18. Freemont PS. The RING finger. A novel protein sequence motif related to the zinc finger. Ann NY Acad Sci 1993;684:174–192.
19. Hochstrasser M. Ubiquitin-dependent protein degradation. Annu Rev Genet 1996;30:405–439.
20. Pickart CM. Mechanisms underlying ubiquitination. Annu Rev Biochem 2001;70:503–533.