Original article

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Juvenile myoclonic epilepsy phenotype in a family with Unverricht-Lundborg disease

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ABSTRACT – *Aims*. Unverricht-Lundborg disease (ULD), an autosomal recessive progressive myoclonus epilepsy, is due to an expansion, or less commonly a mutation, of the cystatin B (*CSTB*) gene. We report a clinical and molecular study of a Tunisian ULD family with five affected members presenting with a juvenile myoclonic epilepsy (JME)-like phenotype.

Methods. The expansion of dodecamers was detected by a deamination/PCR assay. The expression profiles of *CSTB* and other candidate modifying genes, cathepsin B and cystatin C, were established by quantitative RT-PCR, and their respective transcription levels were compared with those from patients with a classic picture of ULD.

Results. Three patients had a fixed phenotype mimicking JME after 29 years of evolution. Only a discrete dysarthria was noticed in the two other patients. No correlation was observed between transcription level and severity of disease.

Conclusion. Genetic screening should be performed in patients with a JME-like phenotype, when careful examination reveals discrete atypical signs of JME. This particular phenotype may be due to modifying genes and/or gene-environment interactions which require further clarification.

Key words: Unverricht-Lundborg disease, juvenile myoclonic epilepsy, cystatin B, cathepsin B, cystatin C

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Unverricht-Lundborg disease (ULD) is the classic and most common progressive myoclonus epilepsy (PME) worldwide (Gargouri-Berrechid *et al.*, 2016). This autosomal recessive (AR) neurodegenerative disease prevails in Scandinavian countries and in the Mediterranean basin, particularly in the Maghreb (Gouider *et al.*, 1998). ULD is caused by an expansion, or less commonly a mutation, affecting the gene encoding cystatin B (*CSTB*), including "CCCCGCCCCGCG" dodecamer repeats localized on chromosome 21q22.3 (Gouider *et al.*, 1998; Genton, 2006; Joensuu *et al.*, 2008; Gargouri-Berrechid *et al.*, 2016). Onset occurs in late childhood or adolescence, with either myoclonus or generalized tonic-clonic seizures (GTCS). During the first five to ten years, myoclonus becomes more severe and disabling, and cerebellar signs develop. The outcome depends on the severity of action myoclonus. This is very variable even in the same family, ranging from minimal impairment to severe handicap in wheelchair-bound or even bedridden patients (Crespel *et al.*, 2016). In some cases, the myoclonus is so mild that it leads to a marked delay in the diagnosis or a misdiagnosis of focal epilepsy or juvenile myoclonic epilepsy (JME) (Kälviäinen *et al.*, 2008; Amroma *et al.*, 2014). Various factors, including some candidate genes, have been suggested to modulate neurodegeneration and consequently the clinical expression of ULD.

We identified a large ULD Tunisian family including five patients, all presenting with a phenotype mimicking JME after a mean duration of 29.6 years. We discuss the clinical, biological, and therapeutic peculiarities of this phenotype. Moreover, we screened *CSTB*, cathepsin B (*CTSB*), and cystatin C (*CST3*) genes as modifying genes that may account for this particular phenotype and compared their transcription levels with those of another family presenting with a typical phenotype of ULD.

Materials and methods

Clinical study

Family 1 (*figure 1*) was ascertained through a proband followed in the neurology department of Razi hospital (Tunis, Tunisia). EEG recordings were performed for three affected members at the initial assessment, then once a year during the follow-up period. The Unified Myoclonus Rating Scale (UMRS)-simplified was used to evaluate the severity of the myoclonus. During follow-up, affected members had a thorough neurological examination every three to six months. The mean follow-up period was 12 years. A detailed genealogy was drawn up, including consanguinity loops. Blood samples were obtained from five affected individuals and their close relatives. All participants or their guardians provided informed consent.

Family 2 (*figure 2*), included four patients with a typical phenotype of ULD. The clinical data are presented in *table 1*.

Genetic analysis

Blood samples were obtained from five affected individuals and their close relatives. All participants or their guardians provided informed consent.

Molecular diagnosis

The expansion of the dodecamer was detected by a deamination/PCR assay (Weinhaeusel *et al.*, 2003). PCR was preceded by the deamination of genomic DNA, converting unmethylated cytosine to adenine. The PCR products were loaded onto a 3,730 sequencing apparatus (Applied Biosystem) and fragments were sized by Gene Scan software using 500 and 1,000-bp ladders.

Quantitative RT-PCR of CSTB, CTSB and CST3

Blood samples from Family 1 (V36, V17, VI1 and V21) and Family 2 (VI1, VI2, VI6 and V9) were collected in PAX gene Blood RNA tubes (QIAGEN). Total RNA was extracted using the MagNaPure apparatus (Roche). cDNA synthesis was performed with 100-ng total RNA using the "Super Script III First-Strand Synthesis Super Mix for qRT-PCR" kit (Invitrogen). Quantitative RT-PCR was performed with Quanti Fast Probe Assays (Qiagen). Levels of messenger RNA expression relative to the *ACTB* gene encoding actin were obtained from a standard curve.

Results

Phenotype

Family 1 resided in a village in which consanguineous marriages were common. *Figure 1* shows multiple complex consanguinity loops, highly suggestive of autosomal recessive inheritance. Five affected individuals from three different sibships were identified. The age at onset was homogeneous, ranging from eight to 13 years. Myoclonus predominating at awakening was the earliest manifestation present at the onset of the disease. GTCS occurred in all patients later. Both myoclonus and seizures were controlled by low doses of valproate and clonazepam. All examined patients had peri-oral reflex myoclonus (PORM), which was correlated to epileptic discharges on EEG (Patients V13 and VI1) (*figure 3*). However, no action myoclonus or fine "postural tremor" were observed. No cerebellar signs were noticed except discrete dysarthria in two patients (V38 and VI1) after a mean duration of 29.6 years. None of the patients had particular

psychiatric symptoms. EEG showed well organized background with no epileptic discharges and no effect from intermittent photic stimulation (V13, V17 and VI1). Three affected patients (V13, V17 and VI1) had somatosensory evoked potentials (SEPs) and cortical reflexes (C-reflexes). All evaluated patients had normal SEPs and negative C-reflexes. All patients were high-functioning and independent. No major

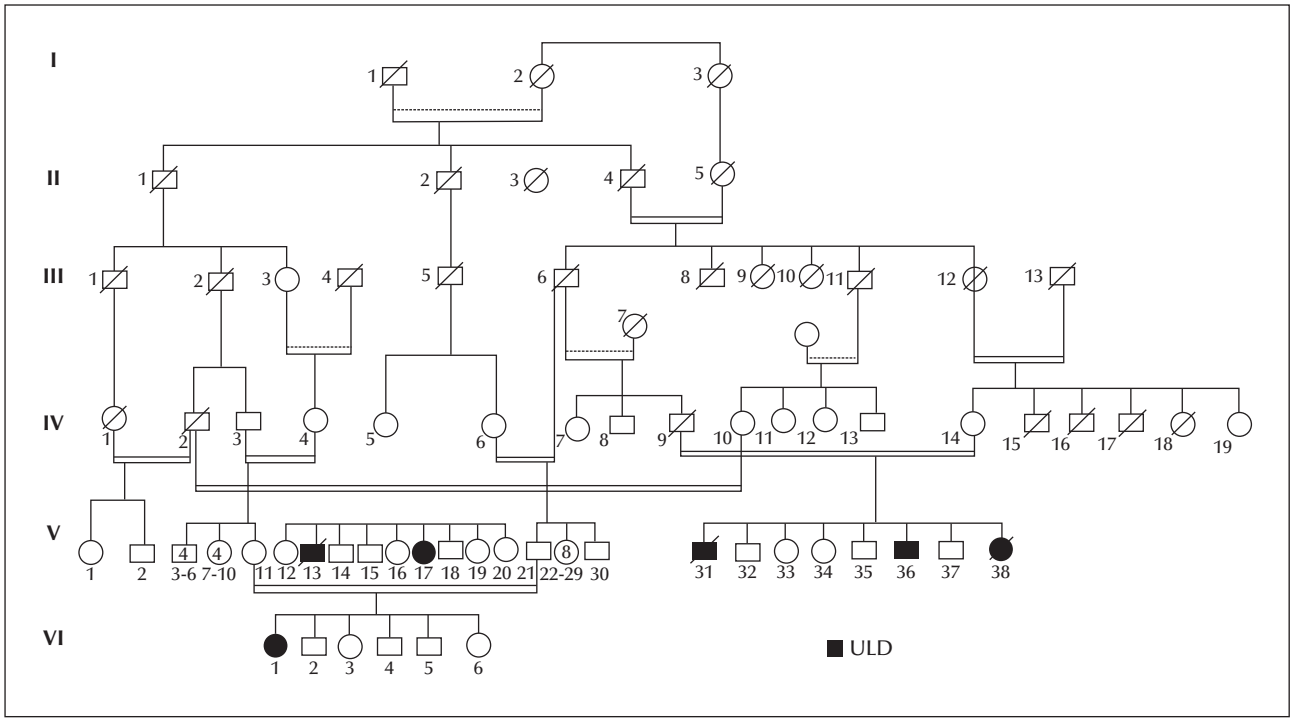

Figure 1. Pedigree of Family 1 with a JME-like phenotype showing multiple complex consanguinity loops and six affected members in three different siblings.

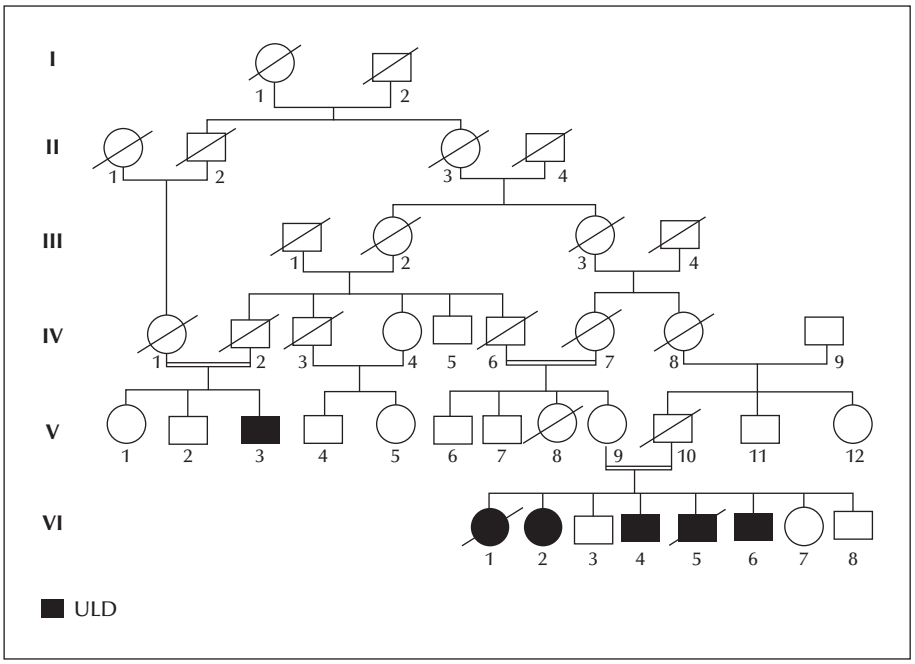

Figure 2. Pedigree of Family 2 with a typical clinical picture of ULD showing affected members in the same siblings.

Table 1. Clinical characteristics of affected members (Family 1 and 2).

Patient	Family 1					Family 2			
	V13	**VI1**	**V17**	**V38**	**V36**	**VI1**	**VI2**	**VI5**	**VI6**
Sex	M	F	F	F	M	F	F	M	M
Year of birth	1977	1984	1984	1960	1972	1970	1972	1978	1984
Age at onset	10	12	8	13	12	11	11	12	10
Disease duration (years)	29	20	24	43	32	35	33	26	22
First symptom	My	My	My	My	My	My	GTCS	My	My
GTCS	+	+	+	+	+	+	+	+	+
Cerebellar signs	-	+[a]	-	+[a]	-	+	+	+	+
IQ	75	94	84	NE	NE	<40	72	74	<40
Antiepileptic drugs	VPA CZP	VPA CZP	VPA CZP	PB	-	PB CZP	VPA PB CZP	VPA PB	VPA PB CZP
Action myoclonus rate/5 (simplified UMRS)	0	0	0	0	0	4	3	3	2

IQ: intellectual quotient; My: myoclonus; VPA: valproate; CZP: clonazepam; PB: phenobarbital; NE: not evaluated; [a]minor dysarthria revealed at clinical examination.

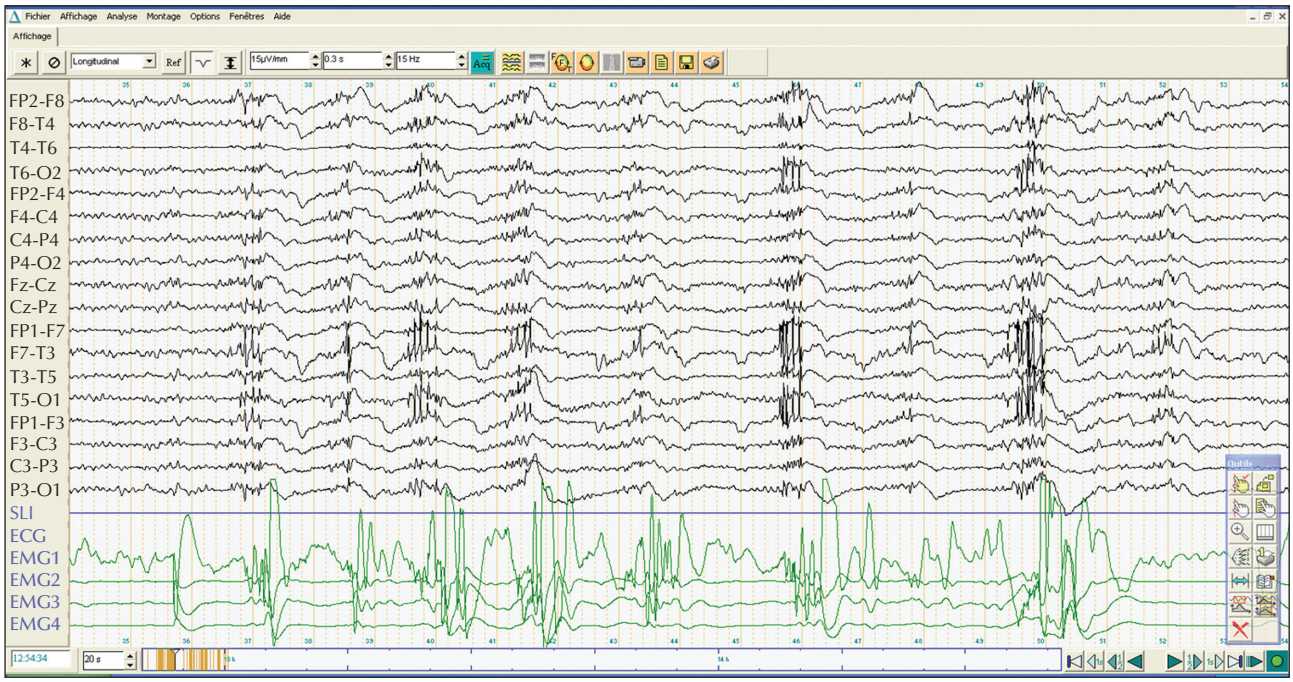

Figure 3. EEG showing generalized epileptic discharges.

cognitive decline was noted, with normal or subnormal intellect.
Family 2 included four patients with a typical phenotype of ULD. *Figure 2* also shows multiple complex consanguinity loops. Mean age at onset was 11 years, mainly with myoclonus. Later on, all of the patients developed GTCS, evident cerebellar signs, and cognitive impairment. Five patients (VI1, VI2, VI4, VI5 and VI6) had neurophysiological assessment showing normal SEPs and negative C-reflexes in all of them. The clinical data are summarised in *table 1*.

Genetic analysis

In Family 1, the five affected individuals carried two expanded dodecamer repeats of the same size, estimated at 58 +/- 1 dodecamers in the promoter region of the *CSTB* gene, confirming the diagnosis of ULD. In Family 2, with a typical phenotype of ULD (*see table 1*), two expanded alleles of 55 and 57 +/- 1 dodecamers were measured in the four patients (*figure 2*).

Transcriptional assays

The expression studies of cystatin B (*CSTB* gene), cathepsin B (*CTSB* gene), and cystatin C (*CST3* gene) are presented in *figure 4*. Based on Q-RT PCR, we evaluated the transcription level of *CSTB*, *CTSB* and *CST3* genes in lymphocytes from the two families: Patients V36, V17 and VI1 and the carrier father V21 in Family 1, and Patients VI1, VI2 and VI6 and their carrier mother V9 in Family 2. For the *CSTB* gene, all patients expressed a low level of transcript (<10%), with very similar levels, however, heterozygous carriers expressed a decreased level compared to controls (about 57% for V21 and 80% for V2). For *CTSB*, the transcription level was very variable between family members, but appeared to remain above the control level in homozygotes as well as heterozygotes; the ratio of patient/control *CTSB* ranged from 1.1 to 1.78. In contrast, the level of *CTS3* was decreased in Patients VI1 (Family 1) and VI2, VI6 and the carrier V9 (Family 2). However, no significant differences could be observed in the transcription level of the three genes between the two families (*CSTB*: p=0.18; *CTSB*: p=0.4; *CST3*: p=0.2; Wilcoxon rank sum test).

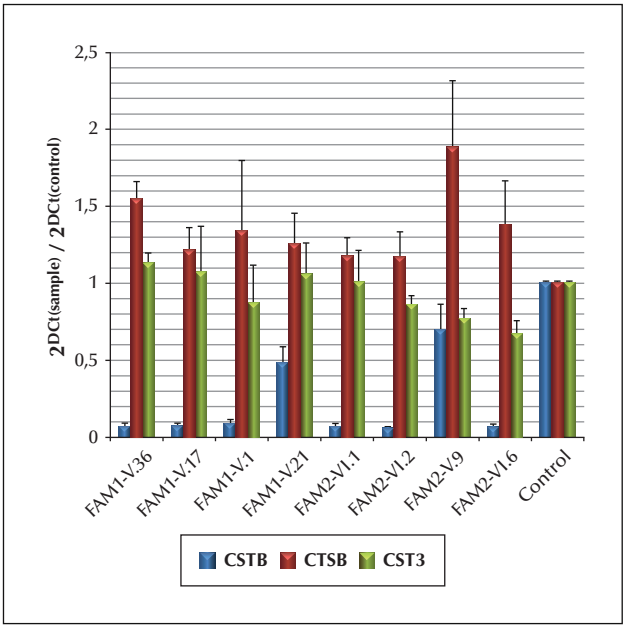

Figure 4. Expression profiles of *CSTB*, *CTSB* and *CST3* genes. The level of transcripts of *CSTB* (blue), *CTSB* (red) and *CST3* (green) was measured by quantitative RT-PCR (q-RT-PCR) in leukocytes from patients and the carrier of Family 1 (Fam1-V.17, V21, VI1 and V21) or 2 (Fam2- VI1, VI2, VI6 and V9) and compared to those of controls. Relative expression (RE) of the sample gene was calculated using the ΔΔCT method using the formula RE = 2ΔΔCT, where CT = PCR cycle in which the sample fluorescent intensity exceeds that of background, ΔCT sample = CT sample - CT ACTB sample, ΔCT control = CT control - CT ACTB control, and ΔΔCT = ΔCT sample - ΔCT control. For each tested individual, the experiment was performed in triplicate. The $2^{\Delta Ct}$ ratio for sample:control for each gene is indicated on the y axis.

Discussion

In the present report, we describe a unique ULD family with a particular JME-like phenotype. Since few isolated cases have been reported with a mild phenotype (Amroma *et al.*, 2014), we present this study of a large family with a JME-like phenotype. This JME phenotype is suspected to be a familial trait and genetically determined.
The proband (V13) of Family 1 had a fixed phenotype mimicking idiopathic generalized epilepsy. After 29 years of evolution, the patient never developed action myoclonus or cerebellar dysfunction and his intellect remained normal. Thus, a diagnosis of JME was first considered. In addition, two other patients remained free of cerebellar signs. Seizures and myoclonus were easily controlled by low doses of valproate and clonazepam. These characteristics matched with JME (Kasteleijn-Nolst Trenité *et al.*, 2013). However, the dysarthria, although discrete, in two affected females put the diagnosis of JME into question and genetic screening confirmed the diagnosis of ULD.
The severity of ULD is now known to be heterogeneous. In a recent Italian study by Canafoglia *et al.*, the authors identified younger age at onset, early severe myoclonus, and seizure persistence as possible predictors of a more severe outcome. They speculated on the genetic determinants of these factors but also greater hyperexcitability responsible for increasing cell damage due to reduced

cystatin B activity (Canafoglia *et al.*, 2017). None of these early predicting prognostic factors were found in our ULD family with JME phenotype. In other studies, it has been previously reported that some ULD patients show a self-limited clinical course (Magaudda *et al.*, 2006; Kobayashi *et al.*, 2011). Moreover, the severity and progression of ULD can vary from one case to another even within the same family (Gouider *et al.*, 1998; Crespel *et al.*, 2016). However, in this family, the phenotype was essentially homogeneous in all affected members and very unusual in terms of clinical presentation, pharmacosensitivity, and evolution. The absence of any cerebellar signs in 3/5 patients after a mean disease duration of 29.6 years represented one of the main clinical peculiarities of this family. The high pharmacosensitivity of myoclonus and the absence of action myoclonus represented the second discrepancy with ULD diagnosis and were the main reasons for the absence of notable functional disability. Concerning PORM, in contrast to PME, these were rare, more difficult to provoke, and epileptic, more consistent with JME (Mayer *et al.*, 2006). In addition, none of our ULD patients with a JME phenotype or a typical ULD form who had SEPs and C-reflex assessment showed neurophysiological features suggesting cortical hyperexcitability. In previous reports, 10 to 20% of patients with JME showed giant SEPs (Salas-Puig *et al.*, 1992), but not all ULD patients are reported to have them (70 to 80%) (Hainque *et al.*, 2018). Hence, neurophysiological examination could contribute to the distinction between JME and ULD. This clinical presentation could be more frequent in Tunisia than in European countries since it was shown that the course of the disease is more severe in Northern Europe than in Maghreb (Gargouri-Berrechid *et al.*, 2016). In addition, Laura Mumoli and colleagues showed an absence of mutation associated with ULD in 33 unrelated patients with JME from Italy (Mumoli *et al.*, 2015).

The size of repeats in Family 1 was within the classic range and even larger than that of Family 2, which does not account for the very mild phenotype. However, it has already been shown that disease severity is not related to the number of dodecamers, suggesting that other factors determine disease evolution (Lalioti *et al.*, 1998). We firstly hypothesized that such factors may influence the level of *CSTB* mRNA, however, the level of *CSTB* transcripts was remarkably decreased in all patients of Family 1 as well as Family 2, with no correlation between functional disability and *CSTB* mRNA level (*figure 4*). By studying the progression of neuronal death in *CSTB*-deficient mice, two proteins were shown to influence neurodegeneration mediated by *CSTB* deficiency: cathepsin B (*CTSB*) and cystatin C (*CST3*). Lehtinen and colleagues showed that cystatin B-deficient neurons were rescued from oxidative stress-induced death by a concurrent decrease in cathepsin B levels (Lehtinen *et al.*, 2009; Polajnar *et al.*, 2013). *CTSB* level was slightly increased in patients of both families, as already reported (Rinne *et al.*, 2002). Kaur and colleagues showed that cystatin C was neuroprotective *in vivo* (Kaur *et al*; 2011). The *CST3* transcript level was not increased in patients of both families. In conclusion, we did not observe any differences in the transcription level of both genes in leukocytes between patients from Family 1 and 2. Although these expression studies were not performed in neural cells, the results support the hypothesis that the JME-like phenotype in Family 1 is due to genetic factors other than *CSTB* itself, *CTSB* or *CST3*, which were the most obvious candidates for modifying genes.

In the current study, none of the ULD patients with a JME phenotype were tested for mutation in genes that are suspected to be involved in JME. In a recent Tunisian study, the authors pointed out the involvement of specific genes including *CACNA1H* and *MAST4* in JME (Landoulsi *et al.*, 2018). However, specific genetic variants that may influence JME susceptibility remain undetermined despite intense research (Santos *et al.*, 2017). The "borderline" phenotypes presented in this report may contribute to a better understanding of JME genetics in the future.

Conclusion

The phenotype of patients in Family 1, caused by classic ULD mutation, is closer to JME than PME even after a long disease duration. Screening should be performed for this ULD mutation in patients with the JME-like phenotype, especially when careful examination reveals discrete atypical signs of JME. This particular phenotype may result from either genetic or environmental factors that influence the effect of the causal recessive mutation during the course of the disease. The identification of these factors will be of great interest and will likely open new therapeutic avenues in the future. □

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None of the authors have any conflict of interest to declare.

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TEST YOURSELF

(1) What is the criterion for a diagnosis of Unverricht-Lundborg disease (ULD) in a patient with juvenile myoclonic epilepsy (JME)?

(2) Does the number of dodecamers within the cystatin B (*CSTB*) gene determine the severity of the ULD phenotype?

(3) What are the most suggestive features of myoclonus in ULD relative to JME?

Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com, under the section "The EpiCentre".