Unverricht-Lundborg disease

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ABSTRACT – We first review the clinical presentation and current therapeutic approaches available for treating Unverricht-Lundborg disease (ULD),
a progressive myoclonus epilepsy. Next, we describe the identification
of disease causing mutations in the gene encoding *cystatin B (CSTB)*. A
Cstb-deficient mouse model, which recapitulates the key features of ULD
including myoclonic seizures, ataxia, and neuronal loss, was generated to
shed light on the mechanisms contributing to disease pathophysiology.
Studies with this model have elucidated the diverse biological roles for Cstb
from functioning as a protease inhibitor, to regulating glial activation, oxidative stress, serotonergic neurotransmission, and hyperexcitability. These
findings set the stage for future studies that may open avenues to improved
therapeutic approaches.

Key words: Unverricht-Lundborg, EPM1, progressive myoclonus epilepsy

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Unverricht-Lundborg disease (ULD)
(EPM1) is the 'purest' type of
progressive myoclonus epilepsy
(PME) (Minassian *et al.*, 2016),
with only minor symptoms associated with epileptic seizures and
myoclonus (Berkovic *et al.*, 1986 ;
Marseille Consensus and Group,
1990). H. Unverricht and H. Lundborg described the condition in
1891 (in Estonia) and 1904 (in
Sweden), respectively (Unverricht,
1891; Lundborg, 1903). The recognition of ULD is still lacking in
areas of low prevalence, moreover, the distribution of ULD
around the world is highly variable
which is due to several factors: i)
unequal distribution of the genetic
defect between different populations; ii) an autosomal recessive
mode of inheritance, which implies
higher prevalence in areas and
cultures with high consanguinity

or a founder effect; and iii) variable availability of modern diagnostic procedures, including molecular biological techniques. Although ULD remains uncommon, it has been increasingly diagnosed among patients with drug-resistant myoclonic epilepsy, as shown, for instance, in Holland (de Haan *et al.*, 2004). However, whereas the most common mutation in ULD affects the cystatin B gene (*CSTB*), no evidence for a role of this gene was found in sporadic or familial cases of juvenile myoclonic epilepsy (JME) (Mumoli *et al.*, 2015). The availability of molecular genetic diagnostic techniques in developed countries has certainly helped.

ULD is still considered a severe and disabling condition, however, recent years have witnessed a shift for ULD, from a very severe, even lethal entity, towards a comparatively bearable disability with little impact, for instance, on life expectancy. Aggravation by phenytoin (PHT) (Eldridge *et al.*, 1983) has contributed to poor prognosis of ULD in countries where PHT is heavily used and titrated to high doses for resistant seizures. The aim of this article is to review the clinical features, prognosis, management, and pathogenesis of ULD.

Clinical characteristics

Onset occurs in late childhood and early adolescence, peaking at around age 12-13. Sex distribution is equal. *Generalized tonic-clonic seizures* (GTCSs) are usually grounds for an initial referral. They occur typically at awakening or during sleep. At onset, GTCS cannot be easily differentiated from those observed in JME, and may occur without prior myoclonic jerks. However, with disease progression, they may evolve into *cascade seizures* (Kyllerman *et al.*, 1991), characterized by a build-up of increasingly intense and violent myoclonic jerks, culminating into a short GTCS; some patients do not report clear consciousness and more or less retain normal contact during this type of seizure. Often, patients experience GTCS or major seizures after a period of progressive increase in myoclonus and subsequently experience less myoclonus, with a decreased risk of major seizures for a period that can last days to weeks; this periodicity has already been described by Unverricht and reported for other PMEs (Ferlazzo *et al.*, 2009; Vanni *et al.*, 2014). Besides awakening, there are no clear triggering factors for GTCSs. Of course, modern drug therapy has contributed to the control of these major seizures, which also seem to decrease spontaneously with advancing age (see below).

Myoclonus is already present in the very early stages, with diffuse myoclonic jerks that predominate at awakening. Over a relatively short period (months to a few years), and in spite of AEDs treatment, myoclonus becomes movement-related and increases with stress. It also becomes physically challenging for patients, *e.g.* patients may fear using stairs or physical strain, and bilateral, violent myoclonus becomes less apparent (unless the patient is challenged or stressed), and partial, erratic myoclonus predominates. Reflex myoclonus, triggered by sensory stimulation (touch, light, *etc.*), initiation of movement, and surprise, is a prominent feature in some patients. Remarkably, myoclonus is less severe or even absent at rest or during sleep. In the early stage of ULD and in patients who are insufficiently treated, myoclonus may fluctuate within the same day (with maximal myoclonus in the morning and in the latter part of the day, when patients are tired) or with an interval of a number of days, sometimes with marked periodicity. These features, however, become less prominent over the subsequent years and with effective drug therapy. Contrary to major seizures, myoclonus does not spontaneously abate in the long term, but may even slowly increase in some adult or middle-aged patients. In the more severely affected patients, myoclonus causes major disability; a wheelchair may have to be used and feeding becomes problematic and slow, especially with the intake of liquids (using a straw may be helpful).

Absence, simple motor, or complex focal seizures may occur, but few video-EEG reports of these seizure types have been documented (Kälviäinen *et al.*, 2008).

Photosensitivity, detected in the EEG laboratory for nearly all patients in the early years of the disease, does not pose major problems in daily life, and tends to abate after 5 to 10 years of disease (Ferlazzo *et al.*, 2007).

Associated neurological symptoms are few. Ataxia, impaired walking, and instability upon standing up are associated with the severity of myoclonus. In our experience, patients cease to be ataxic when myoclonus is fully controlled.

Cognitive impairment may be absent or vary from mild to moderate. Many of our patients reached university level and are qualified professionals. However, whenever possible, ULD patients should avoid professions that involve significant physical activity or fine and precise handling of small objects. Neuropsychological impairment may slightly progress over the years. Early evidence points to a 10-point loss of IQ over ten years (Koskiniemi *et al.*, 1974), and the presence of short-term memory, attention, and executive function impairment is particularly apparent, based on recent studies (Ferlazzo *et al.*, 2009; Giovagnoli *et al.*, 2009).

Psychiatric comorbidities are frequent in ULD but have not been systematically studied. Suicidal behaviour, with loss of interest in life and neglect of therapy,

is a common behaviour, however, only one in 60 of our patients committed suicide. Depression is common and was found in six of eight patients by Chew *et al.* (2008).

Prognosis

The long-term evolution is characterized by limited progression after the first five to ten years (Magaudda *et al.*, 2006), with a varying but fairly stable level of disability thereafter; the outcome in adults ranges from independent active life with minimal impairment to severe disability and wheelchair-bound or even bedridden patients. Early death has a comparatively low incidence and may be due to suicide or accidents, but also to SUDEP, the latter mostly in 'undertreated' patients, in relation to persisting convulsive seizures (Khiari *et al.*, 2009).

Management: diagnosis and genetic counselling

Given the absence of specific clinical or pathological markers, a confirmation of diagnosis of ULD based on genotyping is necessary. Diagnosis used to rely on a combination of positive signs and on the absence of the more specific symptoms and markers of other PMEs, but nowadays can be considered to depend entirely on molecular biological techniques. It is our opinion that this procedure, which remains costly, should be justified by solid electro-clinical evidence, and should not be performed, together with a variety of tests, to screen all possible genetic aetiologies in poorly assessed subjects with epilepsy and myoclonus. The diagnosis of ULD is based on three levels of evidence:

– *1. Clinical evidence*: a combination of history-taking (age at onset, familial and ethnic background, circumstances, and aspects and progression of seizures and myoclonus), examination (including cognitive assessment showing the absence of major and progressive cognitive impairment, and exclusion of associated manifestations such as sensory deficits), and video documentation of myoclonus.

– *2. Complementary evidence*: based on a thorough evaluation of the EEG, polygraphic EEG, and video-EEG recordings (with assessment of changes over time); neurophysiological studies may also help distinguish ULD from other adolescent-onset PMEs, *e.g.* Lafora disease (in which EEG changes are much more spectacular) or juvenile ceroid-lipofuscinosis (with prominent single-flash responses on EEG). A lack of any of the signs and symptoms associated with other PMEs is likely to indicate a diagnosis of ULD, however, other less common PMEs that were recently identified among genetically negative 'ULD' patients may complicate the diagnosis (Franceschetti *et al.*, 2014; Muona *et al.*, 2015)

– *3. Confirmation of diagnosis*: provided nowadays by the demonstration of a pathogenic mutation in both alleles of the *EPM1* gene.

The diagnosis of ULD can be made, or suspected, in various clinical settings. In particular, the most common clinical situation is when idiopathic generalized epilepsy (IGE) or JME has been diagnosed, with a re-assessment of the patient's situation because of an unusual evolution or apparent drug resistance; the clinical work-up should help exclude the possibility of aggravation of IGE by inappropriate AEDs, which may result in a pseudo-PME phenotype.

In some patients with a very likely diagnosis of ULD, the genetic testing of *EMP1* remains negative. Such patients should be discussed with clinicians having experience with PMEs; if the clinical work-up has been thorough, there is usually no need to screen the genes associated with the very typical PMEs which manifest within the same age group (LD, juvenile or adult neuronal ceroid lipofuscinosis, MERRF, DRPLA; *etc*), although recently described genetic defects in *SCARB2/LIMP2*, *KCNC1* or *PRICKLE* might be considered. When the molecular defect remains elusive, and the general condition is very much compatible with ULD, the patient should be diagnosed with ULD (or 'ULD-like PME') and managed as other patients with ULD, with reassessment of the case at two- to five-year intervals by specialized teams.

The diagnosis of ULD should not be given to the family before a final and definitive confirmation, because of the multiple psychological problems that surround the diagnosis of this genetic disease. However, it should be given to the patient and caregivers when confirmed, together with as much information as possible about the condition and its prognosis. Patient organizations can be contacted, and quality information is available on the web. The patients should be encouraged to learn more about the condition and to follow the scientific progress on ULD.

The consequences of a diagnosis of ULD for the family of the proband should not be underestimated. Regarding the prospects, time should be devoted to the change in the patient's and family's lives (see below), but also to counselling on the following topics:

The understandable, expected feelings of guilt and resentment associated with the diagnosis of a genetically transmitted condition should be alleviated, with the use of a few simple statements: inheritance is bilateral (*i.e.* the disease is not transmitted specifically from either the father or mother, but potentially

from both), the disease results from a very uncommon co-occurrence of abnormal genes (even in consanguinous marriages), and subjects with a single abnormal gene will not have the disease.

One major concern is the possible occurrence of ULD in other family members. The risk can be practically excluded in older, fully asymptomatic siblings, but cannot be excluded in *younger* asymptomatic siblings, *a fortiori* in yet unborn siblings, if the parents are young enough to have other children. Whether siblings, especially younger ones, should be referred for molecular screening is still debatable. For *pre*symptomatic ULD cases, there is no reliable, recommended prophylactic treatment to prevent or delay the appearance and progression of symptoms, but this may change in the near future. Having provided all the relevant information, the physician should come to an agreement with the family and obtain their informed consent for all the possible procedures performed for non-affected family members. Concerning future pregnancies for the parents of a patient with ULD, the risks are easy to explain (the risk of ULD is 25%).

The risk of carrying an abnormal gene and transmitting the condition to other generations is also a major concern in families with ULD. Several of our ULD patients have children but none are affected; following medical advice, they had married 'outside the family', an unusual option in some ethnic contexts. Their siblings, or parents (when wanting children with another spouse), as well as other collaterals, may benefit from molecular screening in order to assess the presence or absence of the pathogenic gene found in the proband. An important aspect of diagnosis and genetic counselling is financial and the conditions of insurance and reimbursement (or, more basically, the availability of procedures, tests, and medications) differ greatly between countries and social systems. The families should always be informed about the costs involved; the search for a mutation is expensive, but simple screening for a known mutation is less costly. Similarly, the regulations covering genetic diagnosis, screening, and counselling may differ between countries, and clinicians should always conform to local laws. Obtaining informed consent for the successive steps of diagnostic and screening procedures is a minimum.

Management: medical treatment

This type of PME is characterized by two important features:
– The severity of the condition varies greatly between subjects, even within sibships, and a significant proportion of patients will be able to create a family and lead normal, productive lives with normal or adapted employment, while a significant minority will be severely disabled and dependent upon their family or have an institutionalized life. In a previously published series of 20 ULD patients followed for more than 20 years (Magaudda *et al.*, 2006), eight lived autonomously, six had a family with children, six were normally employed, and seven were dependent on others, including two who were wheelchair-bound. In the largest cross-sectional series (77 subjects) evaluated using modern methodology in Finland, one third of the patients had mild myoclonus, one third had moderate myoclonus, and one third were wheelchair-bound due to severe myoclonus (Koskenkorva *et al.*, 2009).
– The disease has limited progression. Over several (up to 10) years following clinical onset, major seizures and photosensitivity disappear in most patients, while myoclonus stabilizes or progresses only minimally (Magaudda *et al.*, 2006). Thus, a reasonably accurate prognosis can be made fairly early during the course of the condition, 5 to 10 years after onset.

AEDs and piracetam (PIR), a more specific antimyoclonic agent, alleviate the burden of seizures and myoclonus, and their effect is felt throughout the course of the disease; unfortunately, this effect is partial in some cases, as medication does not influence the natural course of the disease. Patients will usually receive an AED after the first GTCS, typically valproic acid (VPA). VPA is normally effective in suppressing, for some time, most GTCS, photosensitivity, and some of the myoclonus. Other AEDs can be used at this stage (Genton *et al.*, 2012); lamotrigine (LTG) is not an AED of first choice in the context of a myoclonic epilepsy, and has been shown to aggravate myoclonus in some patients with ULD (Genton *et al.*, 2006); phenobarbital (PB) and primidone are effective, but produce cognitive side effects on top of the complications attributed to the condition; levetiracetam (LEV) is increasingly used early on in adolescents with IGE, hence in ULD cases, even before confirmation of the diagnosis. Other useful drugs include topiramate (TPM) and zonisamide (ZNS), both with marked antimyoclonic effects. Additional relief can be obtained, often transiently, with benzodiazepines (BZD). The latter (usually clobazam, clonazepam, or diazepam) should be used with care because of a marked initial effect followed by rapid tolerance.

For ULD, the paradoxical aggravating effect of some AEDs may be difficult to assess. There is no evidence that carbamazepine (CBZ), oxcarbazepine (OXC), phenytoin (PHT), eslicarbazepine, gabapentin, pregabalin, vigabatrin or lacosamide are of any benefit. Often, withdrawal of one of these AEDs (especially CBZ or OXC) will bring some relief.

For established ULD, AED treatment leads to polytherapy with a combination of several of the drugs quoted

above (with the exclusion of LTG); the commonly used combinations are VPA+LEV or TPM or ZNS, with an additional BZD (a three- to five-drug combination is quite usual); one can switch between different BZDs in the event of tolerance. In case of transient worsening, with intense myoclonus and serial seizures, there should be no abrupt change in the usual regimen (except for the interruption of a potentially aggravating AED), and IV BZD should be used, as well as, for a limited period, IV PB or PHT.

In practice, some patients will fare reasonably well with a limited drug regimen, while others will remain severely disabled, especially with intractable myoclonus, and will have a much heavier pharmacological load. In such patients, specific therapeutic approaches can be discussed:

– vagal nerve stimulation has been tried with success in some individuals (Smith *et al.*, 2000), including three in our personal experience, but the benefit is limited;

– deep brain stimulation has also been used in PME cases, including some patients with ULD; combined subthalamic and thalamic high-frequency stimulation has brought some relief, especially in the least severely affected cases (Wille *et al.*, 2011). Our personal experience with three patients with a severe form of ULD, who received bipallidal stimulation (as used for dystonia and myoclonic dystonia), was disappointing (Crespel, personal communication).

Management: social support

For ULD, a lifelong condition, social support is at least as important as medical treatment. Psychological support can be provided by patient organizations; one such organization exists in France specifically devoted to ULD, but epilepsy organizations in other countries may offer support. The individual patient should also receive professional psychological support whenever necessary throughout the course of ULD. Physical therapy aims at maintaining a good overall muscular condition and at preserving the ability to walk for the more severely affected patients.

Small amounts of alcohol may temporarily relieve myoclonus (Genton and Guerrini, 1990), but patients should be warned about tolerance, and chronic abuse of alcohol clearly worsens the condition. Photosensitivity, with increased myoclonic jerks, usually abates after several years and is seldom a problem in daily life (patients may watch television or use a computer). Most patients experience increased myoclonus (and an increased risk of major seizures) in the morning, especially after abrupt/sudden awakening, during the active phase of the condition, and should be advised to take their time before getting up. The effect of sleep

deprivation has not been well documented, but anecdotal evidence shows that it may increase myoclonus and seizures, and should be avoided.

At the onset of seizures, the patient is typically in primary or secondary school and experiencing some difficulties with academic requirements, however, it is usually possible to maintain normal schooling. It is useful to discuss with the parents future professional orientation, in light of the possible disability associated with ULD. In most families, the patient will remain at home, but the environment may need to be adapted, *e.g.* avoidance of the use of stairs or ensuring the proximity of a bathroom. Specialized institutions are used for the most severely disabled patients, or for periods to promote education about the condition.

Re-evaluation at a specialized neurological department can be organized at 6- or 12-month intervals, with acute admissions in case of complications, often due to intercurrent diseases (*e.g.* febrile infections). There should be a link between the reference specialized epilepsy team and the local caregiving structure in case of intercurrent health problems, ranging from dental care to surgical procedures. Long-term psychological support is not always necessary, and additional medication (*e.g.* antidepressants) should be discussed with the reference neurologist. Pregnancies have occurred without major complications in less (or moderately) severe women with ULD.

Adult ULD patients usually reach a level of disability and dependency that will remain fairly stable. As stated above, possibilities range from a fully normal life with minimal impairment and monotherapy (usually with VPA) to institutionalized care.

Pathogenesis of ULD: the role of oxidative stress

Oxidative stress is associated with many neurological conditions, including epilepsies (Chong *et al.*, 2005; Kunz, 2002). Several case reports have suggested that antioxidant therapies, including N-acetylcysteine, may alleviate ULD symptoms (Hurd *et al.*, 1996; Selwa, 1999; Ben-Menachem *et al.*, 2000; Edwards *et al.*, 2002). Consistent with the model in which redox homeostasis may be disrupted in ULD, redox analyses in the *Cstb* knockout mouse have revealed that oxidative damage in the cerebellum contributes to the pathogenesis of murine ULD (Lehtinen *et al.*, 2009). Deregulation of antioxidants, including superoxide dismutase (SOD) and the antioxidant glutathione (GSH), are disrupted in the cerebella of ULD mice. The ratio of oxidized to reduced glutathione (GSSG:GSH), a hallmark of oxidative damage, is also increased in ULD mice. Detailed analyses

have uncovered progressive lipid peroxidation in the cerebella of ULD mice. Lipid peroxidation accelerates with age, beginning at baseline in young adult mice (two months of age) and increasing nearly five-fold in six-month-old mice, compared to controls. Evidence for cellular adaptation to accumulating lipid peroxidation was also observed as the activity of glutathione peroxidase, an enzyme that reduces lipid hydroperoxides, increases in ULD mice, compared to controls (Lehtinen et al., 2009).

A hallmark of the ULD mouse model is a progressive loss of cerebellar granule neurons (Pennacchio et al., 1998). Cerebellar granule neurons can be isolated, cultured, and genetically manipulated in vitro (Lehtinen et al., 2006), thus providing a powerful experimental tool for investigating the cellular role of Cstb. In these types of in vitro studies, healthy neurons upregulate Cstb transcription when challenged by peroxide-induced oxidative stress (Lehtinen et al., 2009). Most patients with classic autosomal recessive ULD harbour an unstable dodecamer repeat expansion (5′-CCC-CGC-CCC-GCG-3′) in at least one allele in the Cstb promoter region (Pennacchio et al., 1996; Lafreniere et al., 1997; Lalioti et al., 1997; Joensuu et al., 2008). Introducing a similar expansion into neurons prevents Cstb upregulation during oxidative stress, suggesting that the inability to upregulate Cstb expression impairs normal neuronal responses to oxidative stress. Consistent with this model, reducing Cstb expression by RNA interference (RNAi) or genetic deletion in knockout mice sensitizes neurons to oxidative stress-induced death (Lehtinen et al., 2009).

As part of its downstream signalling, Cstb inhibits the lysosomal protease cathepsin B (Turk and Bode, 1991). Cathepsin B over-expression promotes neuronal death, and cathepsin B activity is upregulated in both Cstb-deficient cells, as well as in ULD patients with CSTB mutations (Rinne et al., 2002). Importantly, the neuronal cell death that occurs upon Cstb loss can be inhibited by a concomitant decrease in cathepsin B both in vitro (Lehtinen et al., 2009) and in vivo (Houseweart et al., 2003), supporting the model that homeostasis related to Cstb-cathepsin B signalling regulates neuronal survival in the mouse model of ULD.

The identification of a role for Cstb in oxidative stress responses in neurons, together with reports that antioxidant therapies alleviate some symptoms of ULD (Ben-Menachem et al., 2000; Edwards et al., 2002; Hurd et al., 1996; Selwa, 1999), provide us with a better understanding of ULD disease pathophysiology. However, whether oxidative damage is a primary cause of disease onset and progression remains to be elucidated. It will be important in future studies to further investigate Cstb-cathepsin B signalling, as well as the downstream consequences of selective oxidative damage to cerebellar lipids. These types of studies may shed light on new approaches to selectively tailor therapies for ULD.

Pathogenesis of ULD: disruption in serotonin metabolism

5-hydroxytryptamine (5HT) metabolism may contribute to ULD pathogenesis. Indeed, an early report identified decreased availability of L-tryptophan (TRP) and its metabolites in cerebrospinal fluid and blood of ULD patients (Pranzatelli et al., 1995). These observations supported the hypothesis that insufficient serotonergic neurotransmission contributes to ULD. Studies using Cstb-deficient mice failed to identify changes in serum TRP concentrations. However, serum levels of 5HT and 5-hydroxyindole acetic acid (5HIAA), an intermediate metabolite of 5HT, tend to be reduced (Vaarmann et al., 2006). These data suggest that the observed disruption in 5HT metabolism in ULD patients may be causally related to Cstb deficiency, rather than being affected by systemic TRP availability or drug therapy. In contrast to TRP concentrations in serum, the brains of Cstb knockout mice have increased levels of TRP and its metabolites (Vaarmann et al., 2006). Kynurenine, the first metabolic intermediate in the TRP kynurenine pathway, is also elevated in the Cstb knockout cerebellum. The myoclonus in ULD is believed to arise from impaired intracortical inhibition leading to secondary hyperexcitability of the motor cortex (Franceschetti et al., 2007). Interestingly, Cstb-deficient mice exhibit significantly higher levels of 5HT and 5HIAA in the cerebral cortex and cerebellum (Vaarmann et al., 2006), the structures where the greatest cellular atrophy and glial appearance have been described (Pennacchio et al., 1998; Shannon et al., 2002). The enhanced serotonergic neurotransmission may result from the loss of GABAergic interneurons, which play a critical role in controlling the serotonergic network in these regions, and are damaged in the Cstb knockout cortex (Franceschetti et al., 2007; Buzzi et al., 2012). While a direct relationship between altered serotonergic neurotransmission and ULD remains to be elucidated, these data suggest that disrupted serotonergic transmission is associated with the disease.

Pathogenesis of ULD: loss of Cstb contributes to defective inhibitory neurotransmission

In addition to its role in redox homeostasis and 5HT metabolism, Cstb protects a cell from endogenous proteases that have the potential to damage neuronal circuitry. Cstb deficiency leads to

hyperexcitability and impaired neuronal function of cortical neuronal networks in both ULD patients and in *Cstb* knockout mice. Several studies have investigated the molecular mechanisms underlying neuronal hyperexcitability by pairing analyses of neurodegeneration with network changes occurring in the hippocampus following kainate treatment, a proconvulsant that triggers excitotoxicity and epileptic events (Arundine *et al.*, 2003). Early *in vitro* hippocampal slice experiments suggested that during kainate perfusion, afferent synaptic activation evokes multiple population spikes in both *Cstb*-deficient and wild-type control mice (Franceschetti *et al.*, 2007). The appearance of such hyperexcitable responses coincides with a rapid decline in the amplitude of the evoked field potentials in slices from *Cstb*-deficient mice. Spontaneous epileptic discharges (SEDs) occur in a subset of slices prepared from wild-type controls, with a delay of about 15 minutes following the onset of kainite perfusion, and persisting until the end of the kainate exposure. In contrast, in *Cstb*-deficient mice, SEDs begin within minutes of kainate perfusion, and progressively decrease in amplitude, ultimately disappearing along with the field responses evoked by electrical stimulation (Franceschetti *et al.*, 2007). To test if increased susceptibility to pro-convulsant agents can be recapitulated *in vivo*, *Cstb*-deficient and control mice received intraperitoneal injections of kainate (30 mg/kg), and their behaviour was recorded for two hours thereafter. Consistent with *in vitro* findings, in this paradigm, *Cstb*-deficient mice display an increased susceptibility to kainite-induced seizures, such that the latency to generalized seizure onset is reduced and the behavioural seizure scores (cumulative seizure score and seizure index) are increased (Franceschetti *et al.*, 2007). To investigate the extent of seizure-induced damage, brain damage was evaluated in *Cstb*-deficient mice and controls one day following kainate administration using several markers of neurodegeneration, including Fluoro-Jade B (Schmued and Hopkins, 2000). The degree of degeneration correlates with the severity of the seizures, and is higher in *Cstb*-deficient mice than in control mice (Franceschetti *et al.*, 2007). In addition, *Cstb*-deficient mice display more neurodegeneration compared to controls with identical seizure scores, suggesting that seizure-induced brain damage is more pronounced in animals lacking *Cstb*. Analyses using immunohistological markers reveal a loss of GABAergic hippocampal neurons, suggesting that the observed hyperexcitability may depend, at least in part, on defective GABAergic inhibition (Franceschetti *et al.*, 2007). To test if ULD is accompanied by a progressive loss of cerebral cortical GABAergic inhibition, cortical GABAergic neurotransmission was analyzed in *Cstb* knockout mice at various ages by histologically visualizing GABAergic nerve terminals, by examining GABA release from isolated nerve terminals, and electrophysiologically evaluating cortical GABAergic tone. While the overall cell numbers are reduced in the *Cstb* knockout cortex, the loss of GABAergic interneurons is more pronounced compared to the general loss of neurons, indicating that GABA interneurons are selectively more vulnerable to kainate-induced damage compared to other neuronal subtypes (Buzzi *et al.*, 2012). A progressive reduction in the density of GABAergic nerve terminals (marked by VGAT staining) is also observed in the sensorimotor cortex of 4-, 8-, and 12-month-old *Cstb* knockout mice. One postmortem ULD patient sample has shown a reduction in cortical thickness and a striking loss of VGAT-labelled GABAergic nerve terminals (Buzzi *et al.*, 2012). Experiments performed in mouse sensorimotor cortex using the paired-pulse paradigm, which is a stimulus protocol to test depression of the conditioned stimulus resulting primarily from GABAergic inhibition, show decreased inhibition in *Cstb* knockout mice at interpulse intervals in cortical layers II-III and V, compared to controls (Buzzi *et al.*, 2012). Perfusion with low concentrations of the GABAA antagonist bicuculline, at 0.5 μM, a concentration suitable for slightly reducing GABAergic-neurotransmission, results in an amplification of the field responses in cortical layers II-III and V of *Cstb* knockout brain slices (Buzzi *et al.*, 2012). Bicuculline leads to a pronounced decrease in depression profile in the paired-pulse protocol in *Cstb* knockout mice, while only minimally affecting control mice (Buzzi *et al.*, 2012). These effects result from the reduction of early GABAergic inhibition occurring simultaneously with the postsynaptic excitatory potential. Taken together, these data support the model that *Cstb* deficiency increases the susceptibility to seizures and to seizure-induced cell death. *In vitro*, hippocampal slices from *Cstb* knockout mice are hyperexcitable, and when perfused with kainate, display precocious and pronounced epileptic-like responses that couple with an early impairment in cellular function. *In vivo*, *Cstb* knockout mice display increased susceptibility to kainate-induced seizures and develop enhanced seizure-induced cell damage with higher degrees of neurodegeneration. The observation of decreased hippocampal GABAergic interneurons suggests that these neuronal subtypes are especially prone to cell damage resulting from *Cstb* loss. The reduction in GABAergic synaptic transmission observed in the sensorimotor cortex implicates a reduction in GABAergic synaptic transmission in ULD. Together, these findings support the model that one key factor contributing to the pathophysiology of ULD is the progressive loss of cortical GABAergic signalling which, with time, leads to hyperexcitability, myoclonus, and seizures.

ULD pathogenesis: precocious microglial activation

Glial activation, and particularly microglial activation, contributes to the mechanisms underlying brain pathologies including neuronal ceroid lipofuscinoses which also display PME (Cooper, 2010). In an early study using aged mice (16-20 months old), GFAP-positive astrocytes were reported to be more abundant in *Cstb* knockout mice than in controls, especially in the hippocampus (Shannon *et al.*, 2002). A recent study including both pre-symptomatic (P14) and symptomatic mice (>one month old), suggests that precocious glial activation is a key mechanism contributing to the pathogenesis of ULD (Tegelberg *et al.*, 2012; Okuneva *et al.*, 2015). Systematic histological analyses using an unbiased stereological approach revealed early and localized glial activation in the brain, as well as in the thalamocortical system. Microglial activation entailed the expression of p-p38 MAPK, a marker of inflammation. While the proportion of pro-inflammatory M1 and anti-inflammatory M2 microglia favours the M2 type earlier in development, the ratio shifts in favour of the M1 type by P30 (Joensuu *et al.*, 2014; Okuneva *et al.*, 2015). The observed microglial activation precedes the onset of myoclonus, and is followed by gliosis and neuronal loss. Interestingly, active microglia undergo morphological changes during ULD disease progression, from that of phagocytic brain macrophages in young animals, to thickened branch processes in older animals (Tegelberg *et al.*, 2012). Consistent with a requirement for microglial activation during disease progression, neuronal loss was not observed in brain regions lacking glial activation (*e.g.* thalamic relay nuclei). These findings are consistent with previous studies suggesting that ULD is a neurodegenerative disease (Pennacchio *et al.*, 1998; Shannon *et al.*, 2002). Indeed, recent approaches using magnetic resonance imaging and diffusion tensor imaging have uncovered white matter degeneration in *Cstb*-deficient mice (Manninen *et al.*, 2014), as well as in patients (Manninen *et al.*, 2015). Taken together, these findings reveal the timing and progression of pathological events in the *Cstb*-deficient mouse brain, highlighting the potential role of glial activation during the initial stages of ULD.

Conclusion

Although nowadays ULD can be treated effectively (albeit only symptomatically) which has led to reduced severity, patients may experience significant disability. A precise molecular diagnostic technique is available. Major progress can be expected in the near future, as elucidation of the mechanisms causing seizures, myoclonus, and associated symptoms (which are mild and mainly cognitive) are likely to bring about pathogenetically-oriented treatment for ULD.

Genetic deletion of *Cstb* in the mouse has provided a powerful tool for modelling ULD in the laboratory. These mice display the triad of symptoms associated with ULD, including myoclonus, ataxia, and neuronal loss. Because *Cstb* is ubiquitously expressed and functions in healthy cells as an inhibitor of the cathepsin family of proteases, it is not surprising that loss of *Cstb* affects a broad range of cellular biological functions, including neuronal death, redox homeostasis, hyperexcitability, and glial activation. The studies reviewed in this article suggest that glial activation may be one of the earliest events contributing to *Cstb*-deficient brain pathology and is accompanied by oxidative stress, neuronal death, aberrant serotonin regulation, and hyperexcitability. Because *Cstb* is ubiquitously expressed, systemic knockout of *Cstb* results in the loss of *Cstb* in all cells of the body. Therefore, a limitation of the present ULD mouse model is the inability to reveal whether the observed phenotypes arise from primary defects in a specific cell type (*i.e.* neurons *vs.* glial cells). It is possible that some observed phenotypes are secondary to defects originating in neighbouring cells.

Collectively, these findings lay the foundation for future studies, which, by harnessing the potential of the ULD mouse model, should improve our understanding of the pathophysiology of ULD and open avenues for tailoring new therapeutic approaches. □

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