## DATA SUPPLEMENTS

### Clinical findings

The disease courses in individual patients are summarized shortly below.

Patient L3 presented between 1 and 2 years of age with myoclonic seizures and drop attacks. His myoclonus presents the characteristic action and postural exacerbation typically seen in PMEs, with a mostly bilateral shoulder distribution, although axial involvement and intermittent eyelid myoclonia are also noticed. No prominent facial dysmorphisms were present. Myoclonic status epilepticus was never seen. The generalized tonic clonic seizures were not characteristically prolonged. Neurological examination revealed mild ataxia that worsened within 3 months, the patient becoming wheel-chair-bound whithin 20 months after onset, when his speech and mental abilities also started to regress. After 6 years of onset patient L3 does not have any speech, is unable to follow commands, and is continuing to have frequent myoclonic daily seizures and right sided brief clonic seizures. He has spastic tetraparesis. Microcephally is not seen and his retinal examination is normal. Valproate helped control the seizures initially, but the seizures recurred and were thereafter resistant to antiepileptic drugs (AEDs).

Patient N2703 presented between 1 and 2 years of age, with myoclonic seizures and started to have tonic-clonic and hypomotor seizures 6-18 months after onset. Progressive mental and motor deterioration also manifested at onset and progressive ataxia appeared approximately 18 months after onset. Four years after onset the patient is non-ambulant and spastic with brisk deep tendon reflexes and a positive bilateral Babinski sign; retinal examination is normal. No Lafora bodies were found in skin biopsy and muscle biopsy was normal. After 8 years of disease progression patient N2703 has severe mental and motor retardation, cannot sit without support, and only recognizes his mother.

Patient N3503 presented with myoclonic seizures between 6-10 months of age. Eight months later she started to manifest progressive motor and mental deterioration. Thirteen years after onset the patient has severe mental and motor retardation and spastic tetraparesis with contractures. Her maximum motor capacity consists in sitting with support. Retinal examination has been normal. She cannot follow objects or voice.

Patient N4103 presented between 1 and 2 years of age with myoclonic and tonic-clonic seizures. Progressive mental deterioration became evident 6 months after onset. Retinal examination at 3 years of age was normal. Two and a half years after disease onset he was hospitalized due to status epilepticus, which was resolved by midalozam infusion. The patient was never able to walk. After 7 years of onset the patient shows severe mental and motor retardation, is unable to hear or follow objects and can only sit with support.

Patient N12604 had delayed speech and motor development and started to manifest progressive ataxia at around 3 years of age. Myoclonic and tonic-clonic seizures manifested 2 years later after which also mental and motor regression were noted. Although she could make sentences at the onset of disease, she has lost her speech ability progressively. She has not had evidence of retinal abnormalities. After 5 years after onset patient N12604 has severe mental and motor deterioration, cannot follow objects or hear and can only sit with support.

Patient N12606 presented between 1 and 2 years of age with myoclonic, and tonic-clonic seizures, after which he started to show signs of motor regression. Ten months later his mental abilities also started to decline. Progressive ataxia appeared at around 3 years of age. Two years and four months after onset the patient has severe mental and motor retardation. He can follow objects and his retinal examination has been normal.

The parents of patient N15103 reported a first generalized tonic-clonic seizure in the patient before 18 months of age. Subsequently they noted mental and motor regression in the child. By 2.5 years of age the patient also had myoclonic and hypomotor seizures as well as hemiclonic simple partial seizures on the right arm and leg with occasional secondary generalization. The myoclonus presented the PME characteristic action and postural exacerbation, being principally confined to the extremities, while no facial dysmorphisms were involved. The generalized tonic clonic seizures were not exceeding 15 min of duration and were thus not characteristically prolonged. About 2 years after onset motor deterioration and ataxia were noted and the patient became wheelchair bound. Three years after onset patient N15103 has microcephaly and severe mental and motor retardation, is bed-ridden and is able to only lift his head. His

retinal examination has been normal. Myoclonic status epilepticus has not been observed. The most useful AED has been levetiracetam.

Patient Pak4 presented below 18 months of age with initially febrile, later afebrile myoclonic seizures and episodes with myoclonic status epilepticus. The myoclonus was increased with action and posture as typically seen in PMEs. The distribution of myoclonus was proximal and distal involving the face, tongue and four limbs. No facial dysmorphisms or hemiclonic seizures were present. The generalized tonic clonic seizures were never characteristically prolonged. During the third year, she developed spastic tetraparesis, ataxia worsened and her speech and mental abilities deteriorated. Eighteen months after onset non-epileptic myoclonus developed (ie myoclonus without spike wave on EEG). However, neither EEG-electromyogram (EMG) back averaging nor somatosensory evoked potentials were performed for the evaluation of cortical reflex myoclonus. Two and a half years after onset the patient has no speech, is unable to follow commands, and is continuing to have frequent myoclonic daily seizures. She is wheelchair-bound, and has acquired microcephaly. For Pak4 the most effective agents were the combination of valproate and clobazam.

Patient Pak5 presented between 1 and 2 years of age with myoclonic seizures of PME characteristic action and postural exacerbation and drop attacks. Similar to his affected sibling (Pak4), he does not have myoclonic status epilepticus, prolonged generalized tonic clonic seizures, hemiclonic seizures, or any facial dysmorphisms. The myoclonus distribution was distal and proximal involving the four limbs, face and tongue. On valproate treatment he developed a hyperammonemic encephalopathy with quick recovery after valproate discontinuation. During the third year his ataxia worsened and his speech and mental abilities also began to deteriorate. Eighteen months after onset the patient has acquired microcephaly, needs assistance to walk, understands easy commands and has a vocabulary of 4 words. He has frequent daily myoclonic seizures. Until present he has not developed non-epileptic myoclonus or a spastic movement disorder. Clobazam was the most helpful AED for Pak5.

**Cell cultures, transfections, and immunofluorescence analysis**

HeLa and COS-1 cells were cultivated in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 5% and 10% fetal calf serum (FCS), 50 mg/ml penicillin, 100 IU/ml streptomycin, and 1× GlutaMAX (Gibco). BHK cells were cultured in Glasgow minimal essential medium (GMEM; Gibco) supplemented with 10% FCS, 50 mg/ml penicillin, 100 IU/ml streptomycin, 1× GlutaMAX, and 5% tryptose phosphate broth (Sigma-Aldrich). HeLa, COS-1, and BHK cells were plated at a density of $2\times10^5$, $2\times10^5$, and $8\times10^4$ cells per well, respectively, onto 6-well plates 24 h prior to transfections.

All transfections were performed with FuGENE® 6 Transfection reagent (Roche). For transfections 1 µg of relevant constructs was used. To arrest protein synthesis, cells were incubated with 50 µg/ml cyclohexamide (Sigma-Aldrich) for 2 h. The cells were fixed with 4% paraformaldehyde (PFA) before processing for immunocytochemistry.

The cytosol was removed in BHK cells via permeabilizing the plasma membrane with 0.05% saponin in cytosolic buffer (80 mM PIPES, pH 6.8, 5 mM EGTA, 1 mM $MgCl_2$) for 3 min.[1] The cells were subsequently fixed with 4% PFA.

For the hippocampal neuronal cell cultures, hippocampi of mouse embryos (SV129-J, Jackson Laboratories, Bar Harbor) were dissected at embryonic day 14 (E14) in ice-cold 100 mM phosphate-buffered saline (PBS, pH 7.4) supplemented with 20 mM glucose. The tissue was trypsinized, and dissociated with a Pasteur pipette. The hippocampal cells were resuspended in Neurobasal medium (Gibco BRL, Invitrogen) supplemented with 1× B27 (Gibco), antibiotics, and 1× GlutaMAX. The cells from two hippocampi were plated in each well of a 6-well poly-d-lysine (Sigma)-coated plate. The culture was maintained for 8 days, after which the cells were fixed with 4% PFA and immunostained.

Cerebellar granule cell cultures, and primary cultures of purified microglia and astrocytes were prepared from postnatal day (P) 5 mice (SV129-J) as described previously.[2, 3]

Following fixation the cells were washed 3 times with PBS and permeabilized with 0.2% saponin in PBS supplemented with 0.5% bovine serum albumin (BSA) for 30 min. The cells were incubated with primary and secondary sera for 1 h at room temperature (RT) and washed with PBS and water. The coverslips were mounted with Gel Mount (Sigma-Aldrich) on object glasses. The immunostainings were visualized using

an Axioplan 2 microscope or a Zeiss LSM 510 Duo confocal microscope (Zeiss, Oberkochen, Germany) and images were obtained with AxioVision 3.1 (Zeiss). For confocal microscopy z-stacks were obtained. Image processing was done using Adobe Photoshop CS4 software (Adobe Systems Inc., San Jose, USA).

**Antibodies**

Mouse anti-HA (1:250; Santa Cruz Biotechnology), rabbit anti-HA (1:500; Santa Cruz Biotechnology) or rabbit anti-KCTD7 (1:200; Sigma-Aldrich), also recognizing endogenous protein, were used for detection of HA-tagged KCTD7. Mouse anti-CD8 (1:200; kindly provided by Dr. Matthew Seaman, Cambridge Institute for Medical Research, Cambridge, UK) was used for detection of the plasma membrane.

Mouse H4A3 (1:100; Developmental Studies Hybridoma Bank, University of Iowa, IA), rabbit CTSD (1:100; Dako), mouse EEA1 (1:100; BD Biosciences), rabbit giantin (1:1000; BioSite), mouse PDI (1:50; Stressgen), and mouse β-tubulin (1:200; Sigma-Aldrich) were used to stain intracellular structures.

In immunohistochemical sections mouse Cdc47 (1:100; NeoMarkers), GFAP (1:100; Dako), F4/80 (1:50; Serotec), NeuN (1:100; Chemicon), and PV (1:1000; Swant), were used for detection of different cell-types. In neuronal cultures mouse SYP (1:200; Dako), PSD-95 (1:100; Transduction Laboratories), and VGAT (1:500; Synaptic Systems) were used against synaptic compartments.

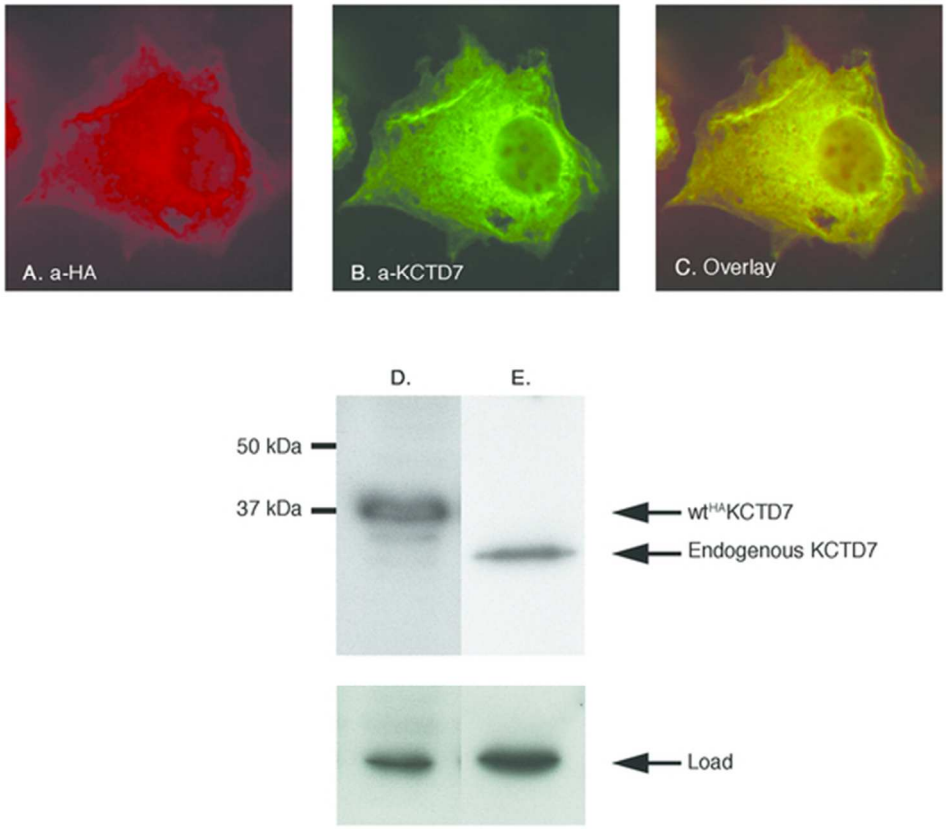
In immunoblotting the primary antibodies were rabbit anti-KCTD7 (1:1000; Sigma-Aldrich), mouse β-tubulin (1:1000; Sigma-Aldrich) and horseradish peroxidase (HRP) conjugated actin (1:1000; Cell Signaling). The anti-mouse IgG (H+L)-Alexa 488, and anti-rabbit IgG (H+L)-Alexa 594 (1:200; Invitrogen) in immunofluorescence stainings, and the HRP-conjugated anti-mouse and anti-rabbit IgG (H+L) (1:3000; Dako) in immunoblotting, were used as secondary antibodies.

**References**

1. **Poupon V**, Stewart A, Gray SR, Piper RC, Luzio JP. The role of mVps18p in clustering, fusion, and intracellular localization of late endocytic organelles. *Mol Biol Cell* 2003 Oct;**14**(10):4015-4027.

2. **Lehtinen MK**, Tegelberg S, Schipper H, Su H, Zukor H, Manninen O, Kopra O, Joensuu T, Hakala P, Bonni A, Lehesjoki AE. Cystatin B deficiency sensitizes neurons to oxidative stress in progressive myoclonus epilepsy, EPM1. *J Neurosci* 2009 May 6;**29**(18):5910-5915.

3. **Giulian D**, Baker TJ. Characterization of ameboid microglia isolated from developing mammalian brain. *J Neurosci* 1986 Aug;**6**(8):2163-2178.




Specificity of the antibody recognizing KCTD7. (A-C) COS1 cells were transiently transfected with HAKCTD7wt. (A, B) The KCTD7 peptides were visualized with either a mouse raised anti-HA antibody (A) or with a rabbit anti-KCTD7 antibody recognizing the protein residues 2-51 (B). (C) Overlay shows full overlap of the stainings obtained with both antibodies. (D-E) Antibody specificity was further validated in Western blot analyses where the overexpressed protein was detected in lysates from COS1 cells transfected with HAKCTD7wt (D) and the endogenously expressed protein in lysates from E14 mouse hippocampal neurons (E). In both lysates KCTD7 was recognized using the rabbit anti-KCTD7 antibody. The expected molecular weight of endogenous KCTD7 is calculated at 35 kDa while that of the HAKCTD7wt construct at 37 kDa. Staining for β–tubulin was used as a control for protein loading.
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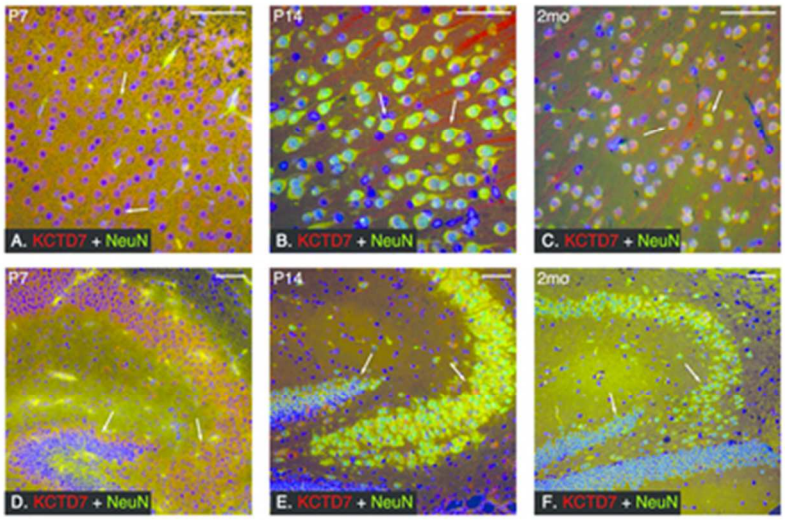


Spatiotemporal expression of KCTD7 in the postnatal mouse brain. In P7, P10, P14, and 2mo mouse brains, KCTD7 was detected ubiquitously throughout the developing brain. (A-F) A prominent expression was detected in the post-mitotic cortical neurons (A-C, white arrows), and the granular and pyramidal cell layers of the hippocampus (D-F, white arrows). NeuN was used as a neuronal marker. NeuN immunoreactivity was weak at P7 but appeared stronger at P14 and 2 mo samples. The scale bars correspond to 20 μm.
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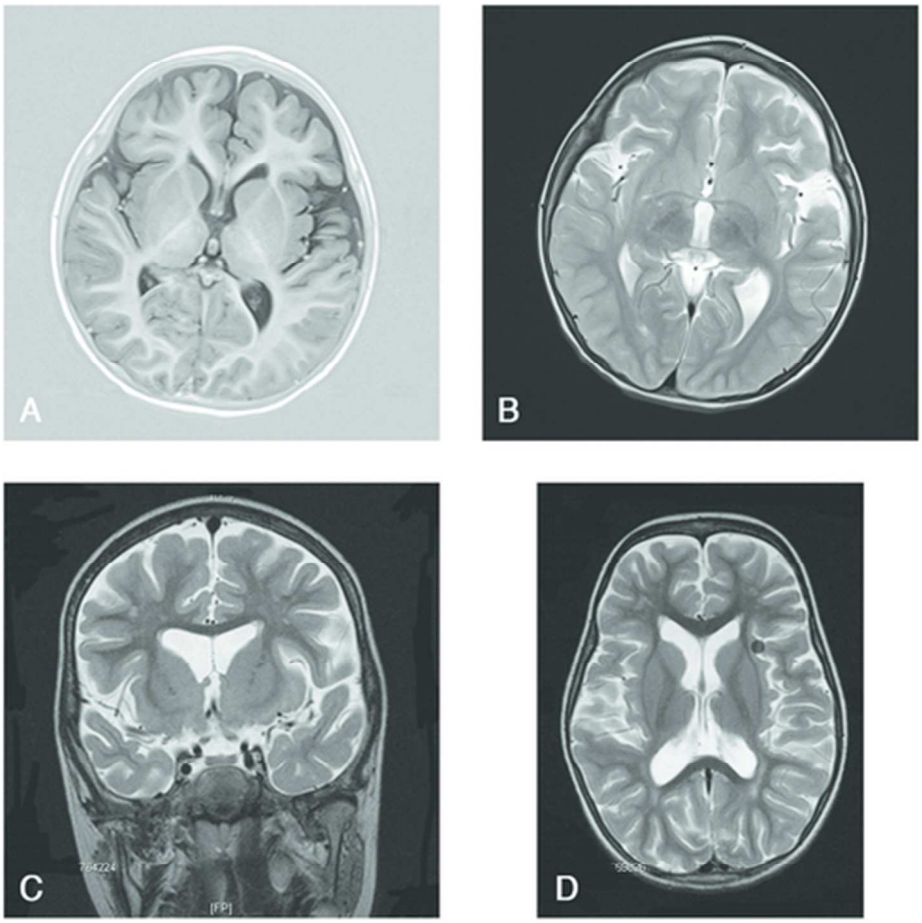
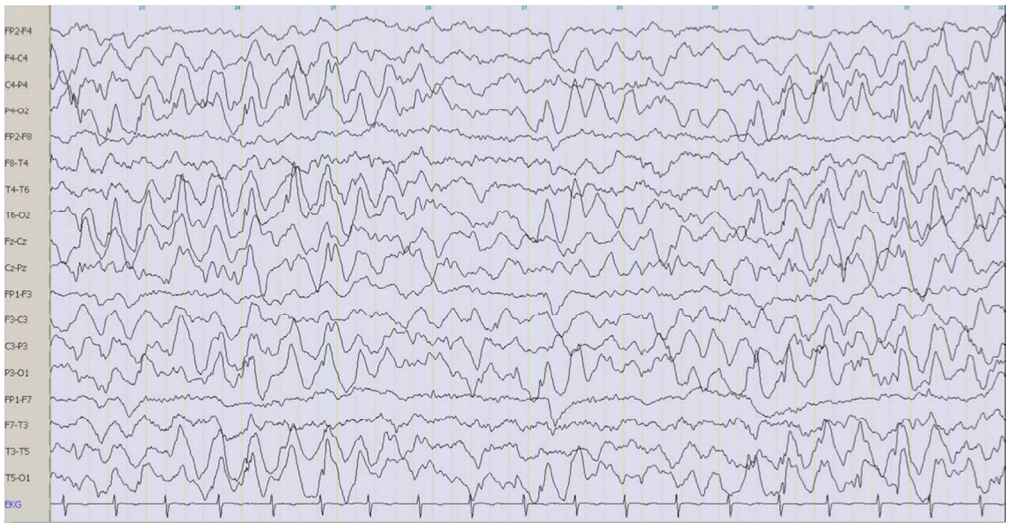



Cranial magnetic resonance imaging (MRI) samples of patients N15103 and Pak4. (A-B) MRI sample of patient N15103 showing frontotemporal cortical atrophy in T1W1 (A) and T2W1 (B) axial images. (C-D) MRI sample of patient Pak4 showing discrete non-specific focal subcortical white matter lesions in coronal (C) and axial (D) T2W1 images.
90x90mm (150 x 150 DPI)




Sequences of rhythmic amplitude delta waves with superimposed spikes predominant in posterior regions from patient Pak4 at 1 year after disease onset.

417x215mm (72 x 72 DPI)