A *de novo* mutation in the Sodium-Activated Potassium channel KCNT2 alters ion selectivity and causes epileptic encephalopathy

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SUPPLEMENTARY INFORMATION

Supplementary Clinical information

The male proband is the only child of non-consanguineous parents with no pertinent family history. He was delivered at term following an uneventful pregnancy, although in retrospect his mother reported fetal hiccoughs. Growth parameters were normal (50-75th centiles). He was hypotonic at birth and had difficulties establishing breast feeding and delayed development. From 3 months, he had multiple daily episodes of staring with eye deviation to the left and single isolated jerks. At 4 months he developed daily clusters of epileptic spasms lasting up to 8 minutes, and there was further regression in his development.

His epileptic encephalopathy has been a lifelong feature (clinical and electrical). At the current age of 4 years, he has multiple daily seizures of mixed semiology that have remained resistant to UKISS trial, a ketogenic diet and the following anti-epileptic medication: topiramate, nitrazepam, levetiracetam, lamotrigine, vigabatrin, ethosuxamide, pyridoxal phosphate and a ketogenic diet. His prominent seizure type is tonic seizures lasting up to 5 minutes; he also has myoclonic jerks and atypical absences. The proband is non-dysmorphic, and his growth parameters, including head circumference, have remained in the normal range. He is hypotonic with truncal instability, has limited visual attention and no verbal responses. He started to walk with significant assistance at the age of 4. He has no other medical conditions or congenital abnormalities.

The electroencephalogram (EEG) has been persistently abnormal with a disorganised background, decrements, multifocal epileptogenic activity or hypsarrythmia (Fig 1A. and B.). Brain MRI demonstrated a generalised reduction in white matter and thinning of the corpus callosum (Fig. 1C. and D.). Extensive neurometabolic and genetic investigations prior to enrolment for research trio exome sequencing were non diagnostic. These investigations included plasma and urine amino acids, urine organic acids, glycosaminoglycans, oligosaccharides and creatine metabolites, serum transferrin isoforms, biotinidase, very long chain fatty acids, α-aminoadipic semialdehyde and Δ^1-piperideine-6-carboxylate, lysosomal enzymology, plasma: CSF (cerebrospinal fluid) lactate and glucose ratios and CSF neurotransmitters. He had a raised glycine ratio on CSF amino acids of uncertain significance but sequencing of the *CLDC* gene was normal, as was sequencing of the *MECP2* and *CDKL5* genes, screening for expansions in exon 2 of the *ARX* gene, and sequencing of mitochondrial

common deletions/duplications and point mutations. Chromosomal microarray detected a paternally derived duplication at 12q21.31q21.32 which was assessed to be likely a benign familial variant.

Exome sequencing analysis

Following filtering, no plausible *de novo*, compound heterozygous, X chromosomal or homozygous variants were identified in any known epileptic encephalopathy genes. A *de novo* non-synonymous variant in KCNT2 was identified, predicted to result in the heterozygous substitution of the normally encoded phenylalanine at position 240 by a leucine (NM_001287819.1:c.[720T>A];p.[Phe240Leu])(=) (Fig. 1C.). The variant was confirmed *de novo* on bidirectional Sanger sequencing of proband and parental samples (Figure 1D).

The affected residue is highly conserved (down to *C.elegans*) and predicted to be pathogenic using the following *in silico* tools: Polyphen (0.904), SIFT (0.04) and CADD (PHRED score 25.5, v1.3). It was not listed in the ExAC database of control individuals depleted for neurodevelopmental phenotypes (last accessed July 2016), or the dbSNP or 1000Genomes database.

Table S1. Primer sequences for mutagenesis

	Forward Primer	Reverse Primer
Phe240Leu rat Kcnt2 in pTRACER	catccccaaagcccacagtagcagtgtcacaatgcagaaataaag	ctttatttctgcattgtgacactgctactgtgggctttggggatg
Phe240Leu rat Kcnt2 in pOX	catccccaaagcccacagtagcagtgtcacaatgcagaaataaag	ctttatttctgcattgtgacactgctactgtgggctttggggatg
Phe240Leu human KCNT2 in psGEM	ccccgaagcccacagtagacagcgtcacaatgcagaaataa	ttatttctgcattgtgacgctgtctactgtgggcttcgggg

Table S1, related to Experimental Procedures: Sequences of primers used to generate Phe240Leu mutations in human SLICK and rat Slick channels

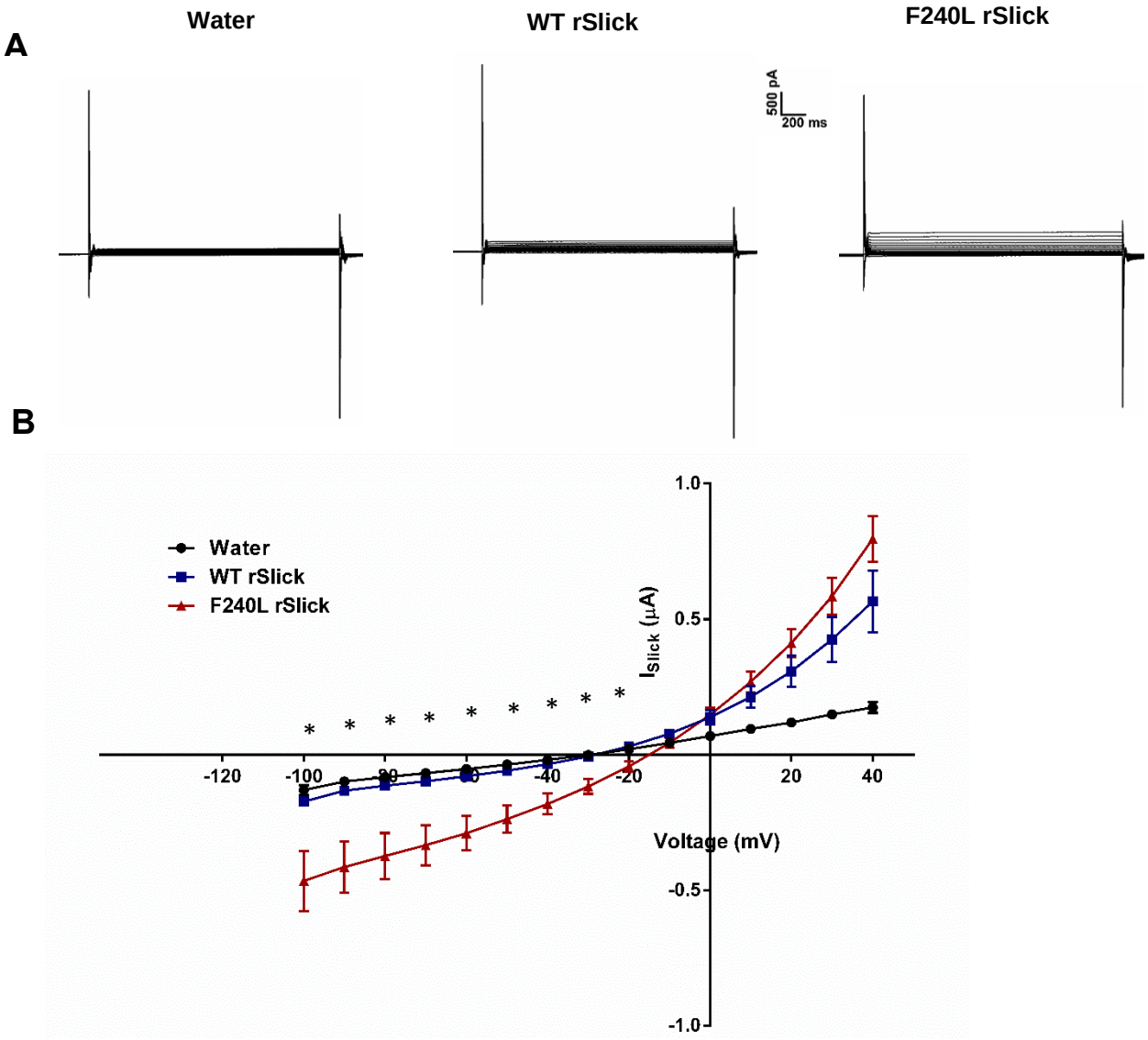

Figure S1, related to Figure 3: Phe240Leu alters K^+ selectivity of rSlick channels in *Xenopus* oocytes. Equal amounts (75 ng) of cRNA for wild-type rSlick, Phe240Leu rSlick or water alone were injected into Xenopus oocytes and two-electrode whole cell voltage clamp was performed 2 days post-injection. **(A)** Representative whole-cell oocyte current traces evoked by voltage steps from -100 mV to +40 mV in 10 mV increments (at a holding potential of -80 mV) in a two-microelectrode voltage clamp setup. **(B)** Effect of the F240L mutation on I_{Slick} current amplitudes recorded in oocytes injected with water, WT rSlick cRNA or F240L rSlick cRNA (n=5 per group per family). Large F240L inward current indicated by significantly higher WT current amplitudes across voltage steps -100 mV to -40 mV via unpaired t-test, *p<.05. Shown are mean ± SEM.