Supporting Information

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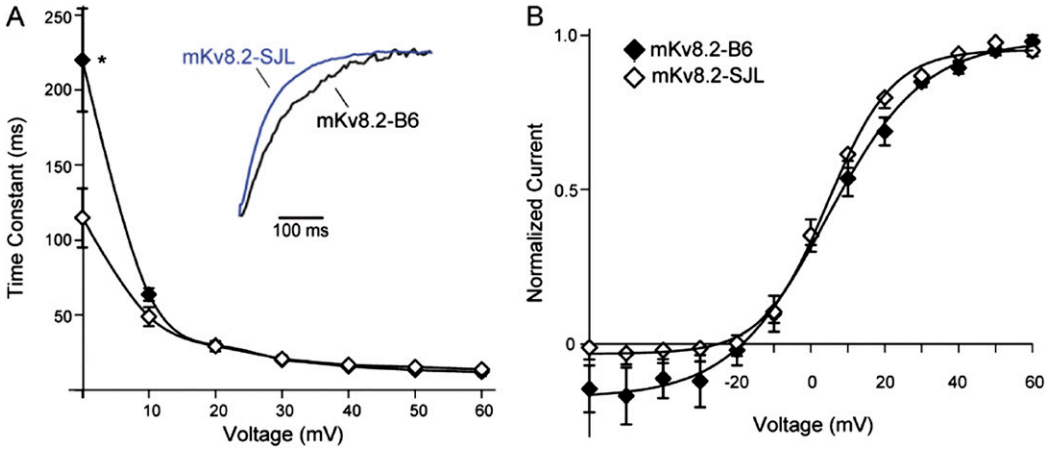

Fig. S1. Biophysical properties of mKv2.1 channels coexpressed with mKv8.2 subunits. (*A*) Voltage dependence of activation time constants for mKv2.1 coexpressed with Kv8.2 isoforms (*$P < 0.05$ compared with WT). Time constants were determined from monoexponential fits to the data. Currents recorded at voltages <0 mV were too small to determine time constants. *Inset:* Averaged current traces (blue, mKv2.1 + SJL-Kv8.2; black, mKv2.1 + B6-Kv8.2) recorded from 10 to 500 ms after a test pulse to 0 mV and normalized to current amplitude measured at 500 ms. (*B*) Voltage dependence of steady-state activation. Tail-current amplitudes were measured at −30 mV after a 2,000-ms activating pulse from −60 to +60 mV. Currents were normalized to peak amplitude and fit with Boltzmann functions. Values for activation $V_{1/2}$ were not significantly different between B6-Kv8.2 and SJL-Kv8.2.

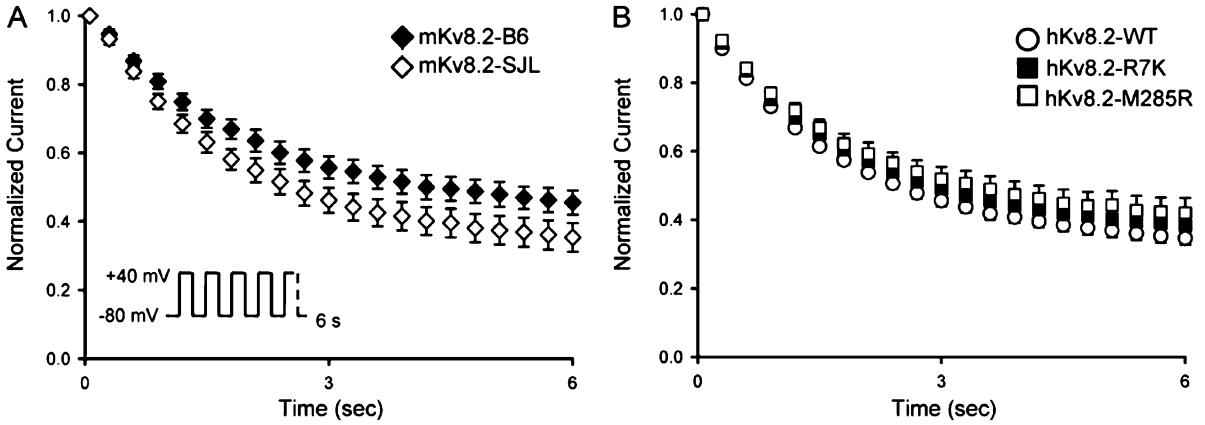

Fig. S2. Cumulative inactivation of Kv2.1 channels coexpressed with Kv8.2. Current was measured during a train of 100 depolarizing pulses to +40 mV of 40-ms duration from a holding potential of −80 mV at a frequency of 17 Hz. Currents were normalized to peak current amplitude. Plotted points correspond to means ± SEM of every fifth pulse for mouse (*A*) and human (*B*) Kv8.2 subunits coexpressed with mouse and human Kv2.1, respectively. Cumulative inactivation kinetics were estimated by monoexponential decay fit to each cell recording and averaged. No significant differences were observed.

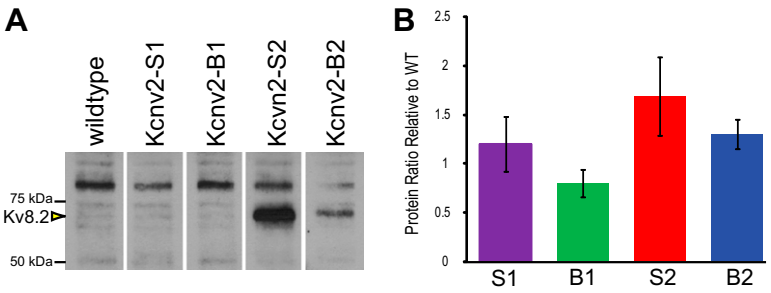

Fig. S3. Relative whole-brain expression of Kv8.2 protein in *Kcnv2* transgenic lines. (*A*) Representative immunoblot of Kv8.2 from *Kcnv2* transgenic mice and nontransgenic littermates (WT). Brain membrane proteins (25 μg) were isolated at 6 wk of age and analyzed by immunoblotting with an affinity-purified rabbit polyclonal antiserum generated against the immunogenic peptide MLKQSNERRWSLSY (ProSci Inc.). The predicted molecular weight of Kv8.2 is 64 kDa (arrowhead). Protein loading is indicated by relative intensity of the nonspecific immunoreactive band at ≈80 kDa. All lanes are from the same exposure of a single blot. Isolation of membrane proteins and Western blotting was carried out as previously described (1). (*B*) Quantitative analysis of Kv8.2 relative protein levels normalized to the 80-kDa band ($n \geq 3$ per genotype).

1. Kearney et al. (2002) Molecular and pathological effects of a modifier gene on deficiency of the sodium channel Scn8a (Na(v)1.6). *Hum Mol Genet* 11:2765–2775.

Fig. S4. Human *KCNV2* variants identified in epilepsy patients. (*A*) Evolutionary conservation of arginine 7. (*B*) Evolutionary conservation of methionine 285.

Table S1. *KCNV2* polymorphisms detected in screening of pediatric epilepsy patient samples

SNP ID	Position* (Mb)	Type	Amino acid	Major/minor alleles	Population minor allele frequency†	Flanking sequence
rs11793555	2717629	5′ UTR	−64 from AUG	G/T	0.333	CTAGAGGCAG(G/T)GAGCAGGTGA
rs7029012	2717698	5′ UTR	−42 from AUG	C/G	0.483	GGACCCCTAC(C/G)ACAGCCAGGA
Unique	2717759	Nonsyn	R7K	G/A	NA	CAGAGTGAGA(G/A)GAGACGGTCC
rs10967705	2717922	Syn	61	C/G	0.459	ACGAAGACGG(C/G)GAGGAGGAGG
rs10967709	2718498	Syn	253	A/G	0.127	TGGAGAAGCC(A/G)TTCTCCTCGG
rs12237048	2718534	Syn	265	C/G	0.415	TCGGGGTGGC(C/G)TCCAGCACCT
rs17656693	2718588	Syn	283	G/A	0.009	ACACCGTGGA(A/G)GAGATGCAGC
Unique	2718593	Nonsyn	M285R	T/G	NA	GTGGAGGAGA(T/G)GCAGCAGCAC
rs7859993	2718654	Syn	305	G/A	0.059	TGGAGCACGT(G/A)GAGATGCTGT
rs7860945	2718717	Syn	326	C/T	0.042	CCACGCCCGA(C/T)CTGAGGCGCT
rs41312842	2729475	Syn	462	C/T	0.097	GCTACGGAGA(C/T)ATGTACCCAG
rs12352254	2729686	Nonsyn	L533V	C/G	0.12	TGAGTGTTTG(C/G)TTGGAAGCAA
rs41306094	2729733	3′ UTR	+6 from stop	C/T	0.097	ATTAGTATTT(T/C)ATAGGACATG

Patient DNAs (n = 209) were screened for *KCNV2* variants by exon amplification, heteroduplex analysis, and sequencing. Two unique, nonsynonymous variants were identified (as noted) in unrelated patients and were subject to biophysical characterization. Nonsyn, nonsynonymous; NA, not available; Syn, synonymous.

*Position is based on the human GRCh37 assembly.

†Population minor allele frequencies are from dbSNP reports.

Table S2. Clinical features of epilepsy patients with unique, nonsynonymous *KCNV2* coding variants

Patient	Seizure types	Age of onset	EEG	MRI	Treatment	Family history	*KCNV2* variant*	Inherited?
1	Partial, febrile and afebrile	2 y	Right temporal epileptiform discharges	Right temporal T2 signal changes	Spontaneous remission: seizure-free at age 10 y with no AEDs	Yes	R7K c.20G > A	Yes
2	Generalized, myoclonic, atonic	2 y	Right centrotemporal spikes; generalized abnormalities	NA	Corpus callosotomy; incomplete control with 3 AEDs	No	M285R c.854T > G	Yes

AEDs, antiepileptic drugs; NA, not available.
*GenBank accession no. BC10135.

Table S3. *SCN1A* and *SCN2A* SNPs in patients with *KCNV2* variants

Gene	Patient	SNP ID	SNP function	cDNA position	Amino acid	Patient genotype	Heterozygote frequency*
SCN1A	1	rs994399†	Intronic	c.965–21C > T		T/T	0.6
		rs9333574	Intronic	c.1171–9_10delTT		ΔTT/ΔTT	0.04‡
		rs7580482†	Synonymous	c.1212A > G	V404	G/G	0.4
		rs6432860†	Synonymous	c.2292T > C	V764	C/C	0.3
		rs2126152†	Intronic	c.2416–37A > C		C/C	0.3
		rs2298771†	Nonsynonymous	c.3199A > G	A1067T	A/A	0.3
	2	rs61741123	Synonymous	c.345T > C	N115	T/C	0.2
		rs994399†	Intronic	c.965–21C > T		C/T	0.6
		rs1542484	Intronic	c.1028+21T > C		T/C	0.5
		rs7580482†	Synonymous	c.1212A > G	V404	A/G	0.4
		rs7559148	Intronic	c.1662+9C > A		C/A	0.2
		rs6432860†	Synonymous	c.2292T > C	V764	C/C	0.3
		rs2126152†	Intronic	c.2416–37A > C		C/C	0.3
		rs2298771†	Nonsynonymous	c.3199A > G	A1067T	A/A	0.3
SCN2A	1	rs67198220	Intronic	c.3399–74+>insG		-/G	0.015
		rs1864885	Intronic	c.4211–31A > G		G/A	0.3
	2	Unique	Intronic	c.385+46C > A		C/A	0.004
		rs67198220	Intronic	c.3399–74+>insG		-/G	0.015
		Unique	Synonymous	c.5326C > T	L1776	C/T	NA

Patients 1 and 2 were screened for *SCN1A* and *SCN2A* mutations by amplification and sequencing of the coding exons. No pathogenic variants were identified. Patient 1 is homozygous for the most common *SCN1A* haplotype (T,G,C,C,A), whereas patient 2 is heterozygous for a rare haplotype (C,A,C,C,A) (1). NA, not available.
*Heterozygote frequencies are from dbSNP reports unless otherwise noted.
†One of five SNPs that define *SCN1A* haplotypes.
‡Heterozygote frequency from Escayg et al. (2001) A novel SCN1A mutation associated with generalized epilepsy with febrile seizures plus—and prevalence of variants in patients with epilepsy. *Am J Hum Genet* 68:866–873.

1. Weiss LA, et al. (2003) Sodium channels SCN1A, SCN2A and SCN3A in familial autism. *Mol Psychiatry* 8:186–194.