95% O_2 and 5% CO_2. Slices were then transferred one at a time to a superfusing chamber for recording. The ACSF contained 119 mM NaCl, 2.5 mM KCl, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 26 mM $NaHCO_3$, 1 mM NaH_2PO_4, 10 mM D-glucose, and 0.1 mM picrotoxin and was equilibrated with 95% O_2 and 5% CO_2. All recordings were made in the CA1 region after removal of the CA3 region. Monosynaptic excitatory postsynaptic potentials (EPSPs) were evoked by stimulating close to the CA1 pyramidal cell layer, and neurons were recorded in bridge mode with sharp microelectrodes containing 2 M potassium acetate (pH 7.3; impedance of 50 to 80 megohms).

5. Depending on the experiment, the microelectrodes contained 5 mM NEM, 1 mM N19 peptide, 1 mM S19 peptide, 0.5 μM botulinum toxin (BoTx), or 0.5 μM heat-inactivated BoTx. We recorded extracellular fields with glass electrodes containing 1 M NaCl (impedance of 5 to 20 megohms) using an Axoclamp-2B amplifier (Axon Instruments) and stimulated Schaffer collateral afferents (100-μs duration) with a bipolar tungsten stimulating electrode. Baseline responses were obtained every 20 s with a stimulation intensity that yielded a half-maximal response. Tetanus-induced LTP was obtained by using a 100-Hz stimulus for 1 s performed four times at 20-s intervals. The magnitude of LTP was measured 50 to 60 min after applying the tetanus. Responses were filtered at 1 kHz, digitized at 4 kHz on a TL-1 interface (Axon Instruments), and collected on a 486 IBM compatible computer. A modification of pClamp software was used for all analyses. Whole-cell, patch-clamp recordings were made with the blind recording technique [M. G. Blanton, J. J. Lo Turco, A. R. Kriegstein, *J. Neurosci. Methods* **30**, 203 (1989)]. Patch pipettes (5 megohms) pulled from borosilicate glass contained 123 mM cesium gluconate, 10 mM CsCl, 10 mM Hepes, 10 mM cesium-EGTA, 8 mM NaCl, 1 mM $CaCl_2$, 2 mM adenosine triphosphate (Mg^{2+} salt), 0.3 mM guanosine triphosphate, 0.2 mM adenosine 3′,5′-monophosphate, and 10 mM D-glucose (pH 7.3, 290 mosM). Stimulus-evoked NMDA currents were recorded at a holding membrane potential of −60 mV in the presence of the non-NMDA receptor antagonist NBQX (10 μM). Baseline values of NMDA EPSCs were obtained from averages of responses during the first 3 min (time 0 on graphs) and defined as 100% for subsequent analyses. Series and input resistances were monitored throughout each experiment with a –3-mV calibration pulse given at a frequency of 0.1 Hz and were typically in the range of 10 to 20 and 150 to 200 megohms, respectively. Experiments were stopped if the series resistance changed more than 15%. The dissolving buffer for SNAP contained 137 mM NaCl, 2.68 mM KCl, 10 mM NaH_2PO_4, 1.76 mM KH_2PO_4, and 250 mM imidazole at pH 7.5. This medium was dissolved 1/26 with the patch pipette solution to give a final concentration for SNAP of 80 μg/ml, then briefly sonicated and placed in the tips of the whole-cell pipettes. The pipettes were then backfilled with the standard whole-cell pipette solution. Baseline values of EPSCs were obtained from averages of responses during the first 2 min (time 0 on the graph) and defined as 100% for subsequent analyses.
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A Potassium Channel Mutation in Neonatal Human Epilepsy

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Benign familial neonatal convulsions (BFNC) is an autosomal dominant epilepsy of infancy, with loci mapped to human chromosomes 20q13.3 and 8q24. By positional cloning, a potassium channel gene (*KCNQ2*) located on 20q13.3 was isolated and found to be expressed in brain. Expression of *KCNQ2* in frog (*Xenopus laevis*) oocytes led to potassium-selective currents that activated slowly with depolarization. In a large pedigree with BFNC, a five–base pair insertion would delete more than 300 amino acids from the KCNQ2 carboxyl terminus. Expression of the mutant channel did not yield measurable currents. Thus, impairment of potassium-dependent repolarization is likely to cause this age-specific epileptic syndrome.

Although most forms of idiopathic epilepsy have a genetic component, only a few specific syndromes are single-gene disorders (*1*). BFNC is an autosomal dominant idiopathic epilepsy characterized by unprovoked partial or generalized clonic convulsions, sometimes with apneic spells, which occur during wakefulness and sleep. Seizures typically start around day 3 of life and most often disappear after several weeks or months (*2*). However, about 10 to 15% of patients have febrile or afebrile seizures later in childhood. Gene loci for BFNC have been mapped to chromosome 20q13.3 (*3*) and to chromosome 8q24 (*4*). Most families in which the disorder occurs are linked to chromosome 20.

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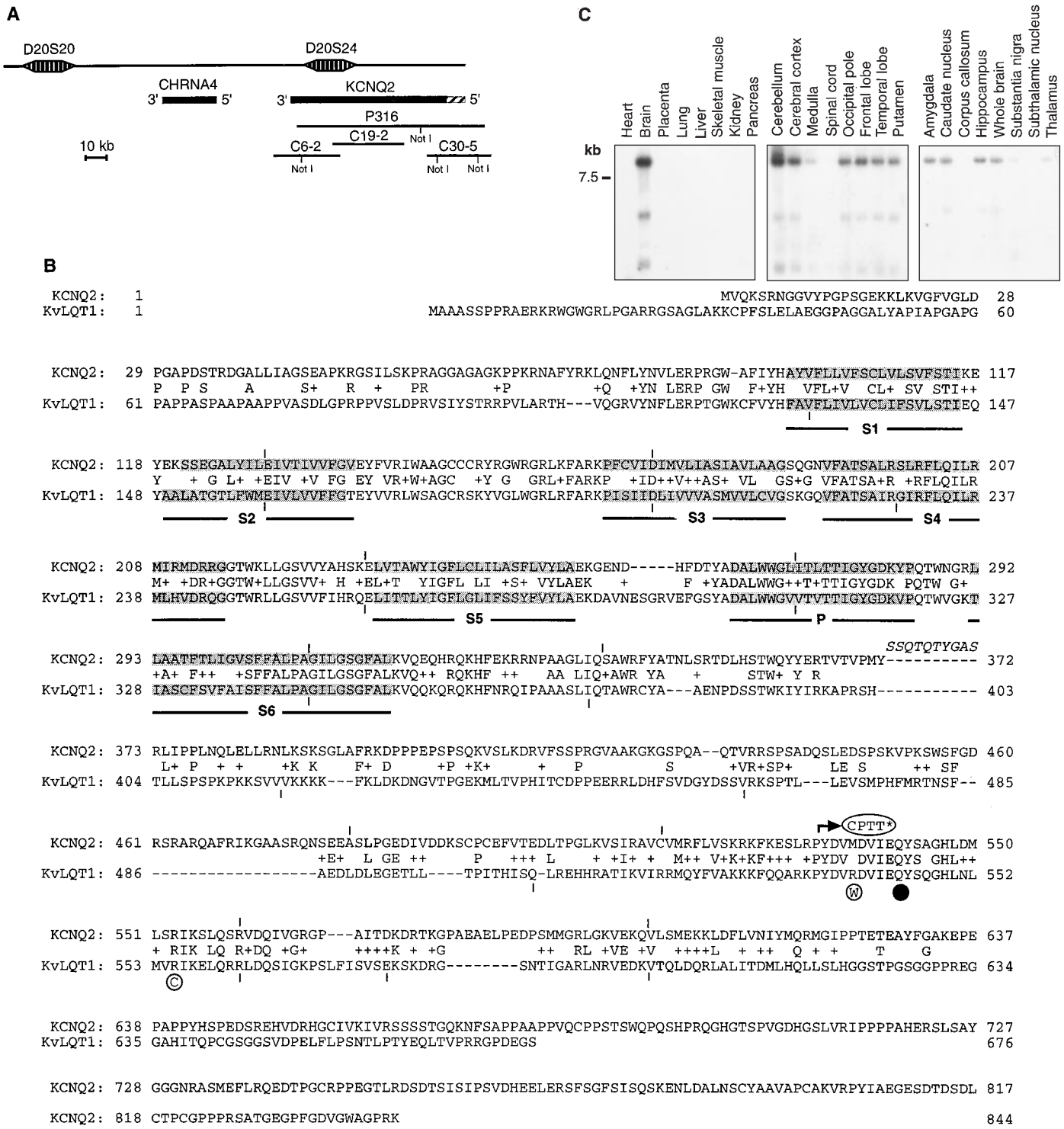

Fig. 1. (**A**) Physical mapping of *KCNQ2*. The localization and orientation of *KCNQ2* with respect to the polymorphic markers D20S20 (RMR6) and D20S24 (IP20K09) are given. *KCNQ2* and *CHRNA4* are separated by approximately 30 kb. *CHRNA4* (α4 subunit of the neuronal nicotinic acetylcholine receptor) has been identified as the gene responsible for autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) in two unrelated families (*26, 30*). Cosmid- (C6-2, C19-2, C30-5) and P1-clone numbering refers to a previously published contig (*5*). (**B**) predicted amino acid sequences (*31*) of KCNQ2 and alignment with KvLQT1 [KCNQ1 (*9*)]. The six potential transmembrane-spanning regions and the P putative pore region are shaded and underlined. Pluses indicate homology between the aligned amino acids. The variable exon of 10 amino acids at position 372 is shown in italics, and its amino acids do not enter the numbering. A similar exon is not present in the published *KVLQT1* sequence. Thin vertical lines indicate the presence of introns in *KCNQ2* (reported here) or in *KVLQT1* (*29*); the list for *KCNQ2* is not complete. The truncated COOH-terminus caused by the 5-bp insertion in the BFNC family is shown above the KCNQ2 sequence. It leads to a truncated channel of 536 residues. This truncation occurs in a region highly conserved between KvLQT1 and KCNQ2. A deletion-insertion mutation (●) in the same region truncates KvLQT1 a few residues downstream in a pedigree with the Jervell and Lange-Nielsen syndrome (*24*). This region of KvLQT1 also harbors missense mutations (indicated by circled W and C below the sequence) in patients with the long QT syndrome (*23*). (**C**) Tissue distribution of KCNQ2. Northern blot analysis (*32*) of polyadenylated RNA from multiple human tissues (left) and from different human brain regions (center and right).

Using cosmid DNA from a previously described contig in the chromosomal region 20q13.3 (Fig. 1A) (5), we isolated a 3.4-kb partial cDNA clone from a human fetal brain cDNA library and extended it by 4 kb toward the 5′ end by RACE (rapid amplification of cDNA ends) experiments (6). Searching the GenBank database revealed that the 5′ open reading frame is roughly 50% identical to KvLQT1, a potassium channel mutated in the long QT syndrome (*7*), and a 1160–base pair (bp) stretch is identical to a partial cDNA isolated previously (8). The *KVLQT1* potassium channel gene will be renamed *KCNQ1* (*9*), and we name the present homolog *KCNQ2*. Interestingly, we have also identified (*10*) another *KCNQ* homolog (*KCNQ3*) on human chromosome 8q24 close to the second locus (*4*) for BFNC.

The complete open reading frame of *KCNQ2* encoded a protein of 844 amino acids, with the hallmarks of K^+ channels defined by the *shaker* channel in *Drosophila melanogaster* (Fig. 1B) (*11*). Similar to KvLQT1, the transmembrane block with six putative transmembrane domains S1 through S6 was followed by a long cytoplasmic COOH-terminus. In addition to a 70% identity in the transmembrane block, there was a conspicuous homologous region in the cytoplasmic COOH-terminus. A splice variant isolated from adult brain had an additional short exon encoding 10 amino acids roughly 50 residues COOH-terminal from S6 (Fig. 1B). Northern (RNA) analysis indicated that KCNQ2 was specifically expressed in the brain (Fig. 1C). KCNQ2 message was widespread in areas containing neuronal cell bodies, but low in the spinal cord and in the corpus callosum, which contains primarily axons and glia.

Seizures are characterized by paroxysmal neuronal hyperexcitability. Ion channels that regulate neuronal excitability have been proposed as possible epilepsy genes (*12*). *KCNQ2* was therefore an excellent candidate gene for BFNC. We partially determined its genomic structure (*13*) and screened (*14*) a large Australian Caucasian pedigree (Fig. 2A) previously linked to chromosome 20q13 (*15*) for mutations in *KCNQ2*. We identified a 5-bp insertion at the triplet encoding amino acid 534 in a segment highly conserved between KCNQ2 and KvLQT1 (Figs. 1B and 2B). The resulting frameshift would result in a premature stop, which would truncate more than 300 amino acids. This insertion cosegregated with BFNC but not with febrile convulsions in the pedigree (Fig. 2A) and was not found in a control panel of 231 independent Caucasian blood donors.

We then examined the functional effects of this mutation as further support for its causative role in BFNC. *Xenopus laevis* oocytes injected with *KCNQ2* complementary RNA (cRNA) displayed a current (*16*) that slowly activated at voltages more positive than –60 mV and was fully activated at 0 mV (Fig. 3, A and B). The open channel was slightly inwardly rectifying. Ion substitution experiments (Fig. 3C) indicated that the current was potassium selective and had a K > Rb > Cs > Na permeability sequence. These currents resembled those of KvLQT1 in their permeability sequence, voltage dependence, and kinetics (*17*–*19*).

When we expressed the truncated KCNQ2 protein, we could not detect currents differing from negative controls, indicating that the mutation abolished channel function. We then coinjected mutant and wild-type (WT) cRNA at a 1:1 ratio to mimic the situation in a heterozygous patient. Currents were reduced when compared to those recorded from oocytes that were injected with the same total amount of WT cRNA (Fig. 3, D and E). There was no obvious dominant negative effect, however, but haploinsufficiency may be enough to explain the dominant mode of inheritance of this disorder, which generally occurs transiently during infancy. Moreover, differences between expression systems and the in vivo situation are not uncommon and may be due to differences in protein stability or trafficking. An attractive alternative hypothesis is that the KCNQ2 channel protein normally interacts with a β subunit, and that its absence in the oocyte explains the failure to exhibit an obvious dominant negative effect. The homologous KvLQT1 channel associates (*17*–*19*) with IsK (also known as minK) (*20*), which significantly

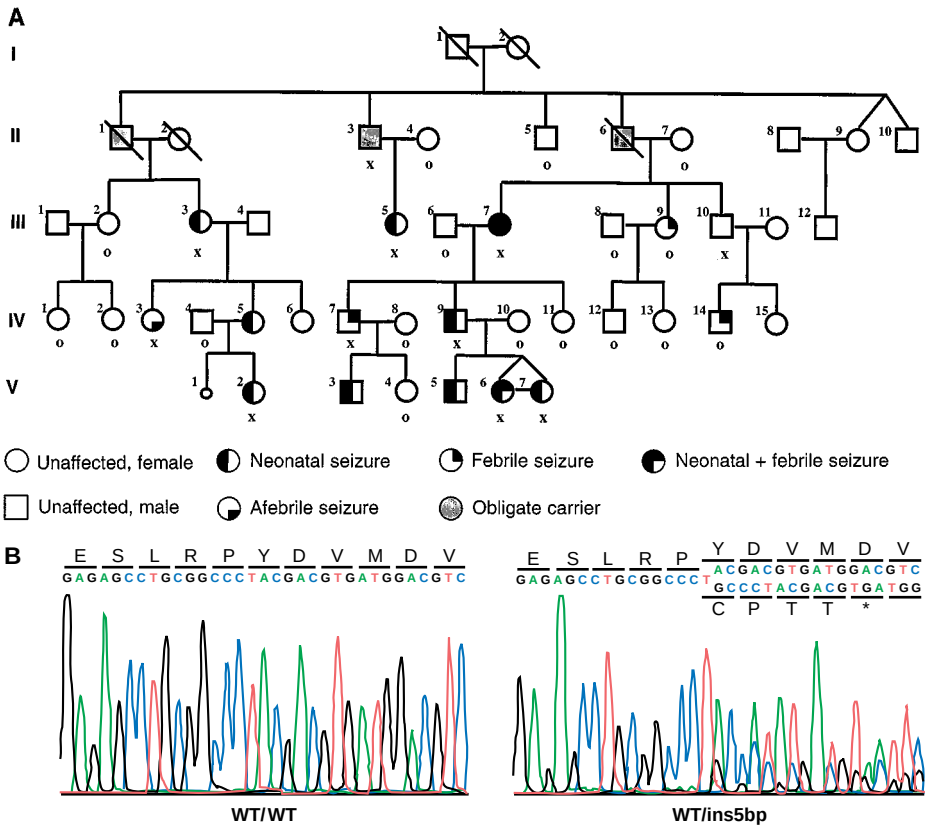

Fig. 2. (**A**) Segregation of the mutated allele in the Australian pedigree with BFNC. Since the first description of the family (*15*), individual IV-14 has been newly diagnosed to have febrile convulsions. He is homozygous for the wild-type allele, implying that febrile convulsions are not caused by the *KCNQ2* mutation, but represent a different phenotype. Individual III-10 is heterozygous for the mutation, but not affected. This could be explained by the reduced penetrance (~80%) of the disease. x, individuals carrying the 5-bp insertion; o, individuals with two normal alleles. Unmarked individuals were not typed. A slash through symbols indicates a deceased individual. (**B**) *KCNQ2* mutation identified in this pedigree. For comparison, genomic sequences from an unaffected control (WT/WT) and of index patient III-5 (WT/ins5bp) are shown. The nucleotide sequences and translations are shown above the direct sequencing traces from amplified exons. The insertion of five nucleotides (which can be explained by a duplication) causes a frameshift that results in a premature stopcodon. The index patient is heterozygous for this insertion.

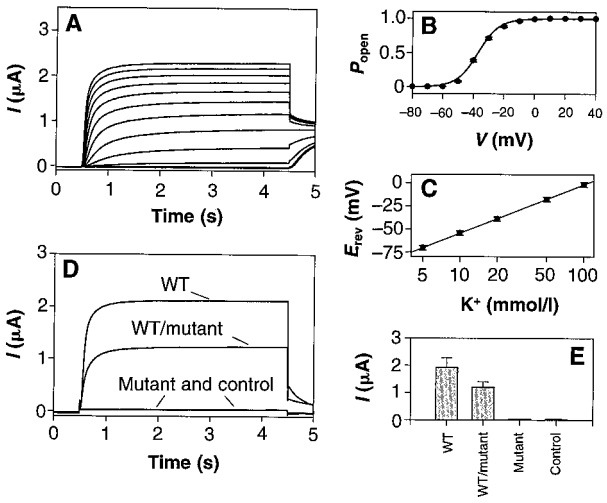

Fig. 3. Electrophysiological analysis of KCNQ2 and its mutant in *Xenopus* oocytes. (**A**) Two-electrode voltage clamp traces of an oocyte expressing KCNQ2. From a holding potential of −80 mV, the oocyte was clamped for 4 s to values between −80 and +40 mV in steps of 10 mV, followed by a constant pulse to –30 mV. (**B**) Open probability (p_{open}) as a function of voltage, as determined by tail current analysis. Half-maximal p_{open} is at –37 ± 2 mV, and the apparent gating charge is 3.7 ± 0.4 (n = 12, ±SD). (**C**) Shift of reversal potential with the external K concentration (n = 8); the shift (53 mV per decade) indicates a channel predominantly selective for K^+. Substitution of external K^+ by other cations yields the following permeability ratios: P_K/P_{Rb} = 1.27 ± 0.01; P_K/P_{Cs} = 7.4 ± 0.5; and P_K/P_{Na} = 51 ± 4 (±SEM, n = 9). (**D**) Current traces of WT KCNQ2, a 1:1 coinjection of WT and mutant KCNQ2 (WT/mutant), mutant KCNQ2, and mock-injected control oocytes. The traces for mutant KCNQ2 and control oocytes cannot be distinguished. From a holding potential of −80 mV, the voltage was clamped for 4 s to +20 mV. Except for the last set of experiments, the same total amount of cRNA (5 ng) was injected into single oocytes. (**E**) Mean currents (after 4 s at +20 mV) averaged from several experiments as in Fig. 3D. The error bars indicate SEM (n = 5 to 10).

alters its currents, and mutations in IsK can also lead to cardiac arrhythmias (*21*). By contrast, when we expressed KCNQ2 together with IsK, currents did not seem to differ significantly from a linear superposition of KCNQ2 currents with IsK currents (*22*) [stemming from *Xenopus* KvLQT1/IsK heteromeric channels (*18*)]. Given the high homology of KCNQ2 to KvLQT1, it is tempting to speculate that there may exist an IsK-like β subunit for KCNQ2 as well, and that mutations in its corresponding gene may be responsible for this or other forms of epilepsy.

Within the long COOH-termini of both KCNQ2 and KvLQT1, there is a highly homologous region. The KCNQ2 truncation found here, as well as some *KVLQT1* mutations leading to the long QT syndrome (*23*) [including a truncation (*24*)], occur exactly in this region (Fig. 1B). Thus, this part of the protein is likely to serve an important, as yet unknown, function.

Potassium channels are important for repolarizing action potentials. Mutations in the KCNA1 potassium channel cause episodic ataxia, a nonepileptic disorder with paroxysmal cerebellar symptoms, although seizures occur in a few cases (*25*). Because BFNC is associated with the loss of function of a potassium channel, the pathological neuronal hyperexcitability in this epilepsy syndrome is likely to be caused by impaired repolarization. Support for the emerging concept of the idiopathic epilepsies as ion channel disorders comes from our previous observation of a nicotinic acetylcholine receptor subunit defect in a form of human partial epilepsy (*26*) and of calcium channel defects in certain inherited forms of epilepsy in mice (*27*). No other gene defects have yet been identified in human idiopathic epilepsies.

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6. A human fetal brain cDNA library (Clontech) was screened with the insert of cosmid C19-2 (*5*), and a single clone was isolated (insert size: 3.4 kb). It was extended in seven consecutive steps with gene-specific primers, using the 5′ RACE kit (Life Technologies) with adult human brain cDNA. Sequences were validated by sequencing independent RACE clones. The resulting composite sequence differed at the 3′ end from HNSPC, a previously reported partial cDNA (*8*). This may be due to an unspliced intron at the 3′ end of the HNSPC clone. Using an appropriate 5′ primer and a 3′ primer after the stop codon, we amplified a full-length cDNA from a human fetal brain cDNA library (Clontech) and subcloned it into the expression vector PTLN (*28*). The cDNA sequence of *KCNQ2* has been deposited in the European Molecular Biology Laboratory–GenBank database (accession number Y15065).
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14. Using intronic primers, single exons were amplified from genomic DNA by PCR. These were either sequenced directly with an ABI 377 sequencer or were first checked by single-strand conformational analysis and heteroduplex analysis as described (*30*).
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16. The expression vector PTLN (*28*), containing wild-type or mutant KCNQ2 or ISK (*19*), was linearized with Mlu I. Capped cRNA was transcribed, using the Sp6 RNA polymerase in the mMessageMachine kit (Ambion). The 5-bp insertion of the BFNC pedigree was inserted into the cDNA by recombinant PCR. All constructs were fully sequenced. About 5 ng of cRNA were injected into *Xenopus* oocytes prepared as described (*19*). After 2 to 4 days at 17°C, the oocytes were examined by two-electrode voltage-clamping in saline containing 98 mM NaCl, 2 mM KCl, 2.8 mM $MgCl_2$, 0.2 mM $CaCl_2$, and 5 mM HEPES (pH 7.4). For ion substitution experiments, NaCl and KCl were partially substituted by equivalent amounts of KCl (for NaCl), RbCl, or CsCl. Expression studies were performed with the variant lacking the additional exon (Fig. 1B). KCNQ2 that had this additional exon gave similar currents.
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31. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
32. Human Northern blot membranes (Clontech) were hybridized with a ^{32}P-labeled 430-bp fragment of *KCNQ2* (encoding amino acids 144 through 288), using protocols provided by the manufacturer, and the membranes were exposed to photographic films (Kodak XR).

33. We thank J. Stoodt and P. Hausmann for excellent technical assistance, G. A. Nicholson and M. L. Kennerson for the original mapping of the pedigree to 20q13 (*15*), and M. M. Nöthen for the controls. Supported by grants from the Deutsche Forschungsgemeinschaft to O.K.S. (Ste769/1-1; SFB 400/B5) and to T.J.J. (Je164/3-1), the Fonds der Chemischen Industrie to T.J.J., and the National Health and Medical Research Council to S.F.B.

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