

- 95% O₂ and 5% CO₂. 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 CaCl₂, 1.3 mM MgSO₄, 26 mM NaHCO₃, 1 mM NaH₂PO₄, 10 mM D-glucose, and 0.1 mM picrotoxin and was equilibrated with 95% O₂ and 5% CO₂. 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 μ M botulinum toxin (BoTx), or 0.5 μ M 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- μ s 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 CaCl₂, 2 mM adenosine triphosphate (Mg²⁺ 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 μ M). 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 NaH₂PO₄, 1.76 mM KH₂PO₄, 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 μ g/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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 27. We thank H. Czerwonka for secretarial assistance. P.-M.L. was supported by CNRS and by a North Atlantic Treaty Organization fellowship. R.A.N. is a member of the Keck Center for Integrative Neurobiology and the Silvio Conte Center for Neuroscience Research. R.C.M. is a member of the Center for Neurobiology and Psychiatry and the Center for the Neurobiology of Addiction. R.A.N. is supported by grants from the NIH. R.C.M. is supported by grants from the NIH, the Human Frontier Science Program, and the McKnight Endowment Fund for Neuroscience.

5 September 1997; accepted 25 November 1997

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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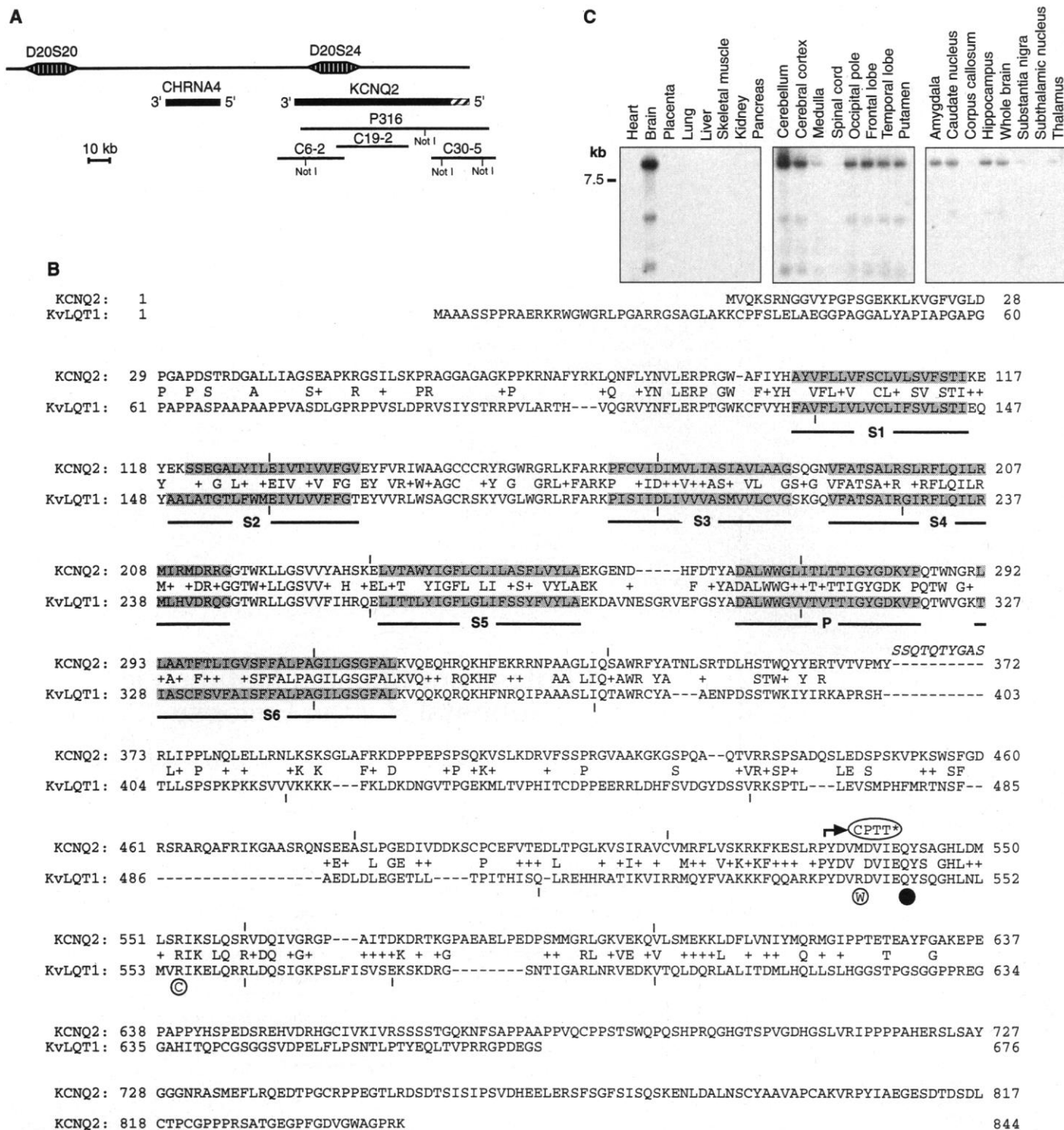


Fig. 1. (A) Physical mapping of *KCNQ2*. The localization and orientation of *KCNQ2* with respect to the polymorphic markers D20S20 (RMR6) and D20S24 (IP20K09) are given. *KCNQ2* and *CHRNA4* are separated by approximately 30 kb. *CHRNA4* ($\alpha 4$ subunit of the neuronal nicotinic acetylcholine receptor) 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

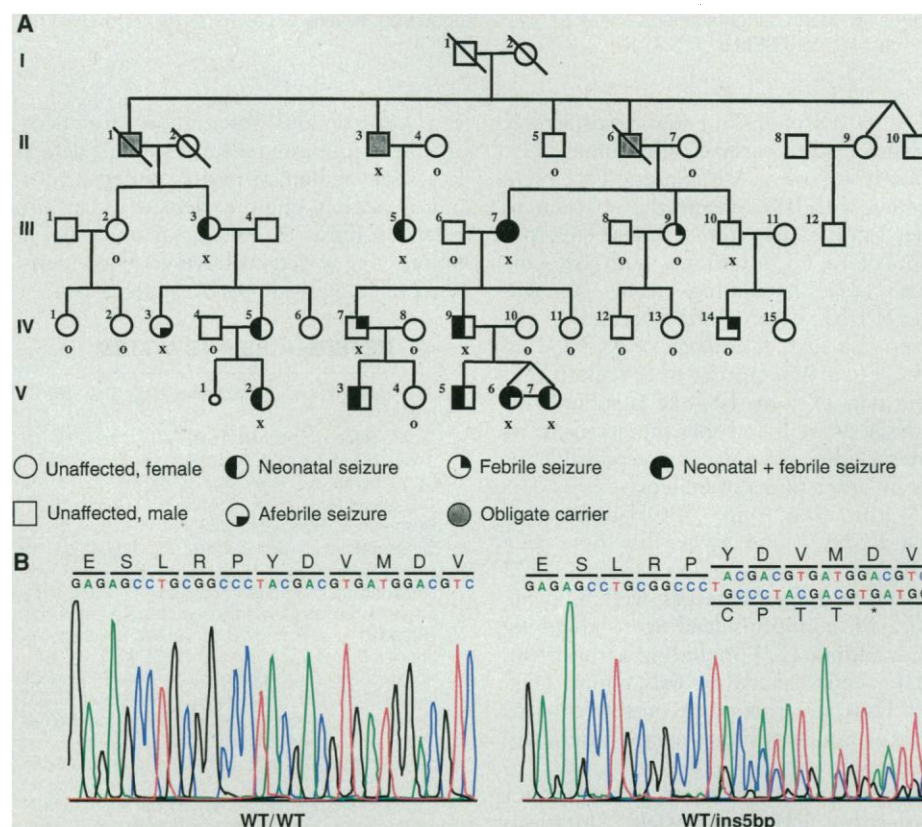
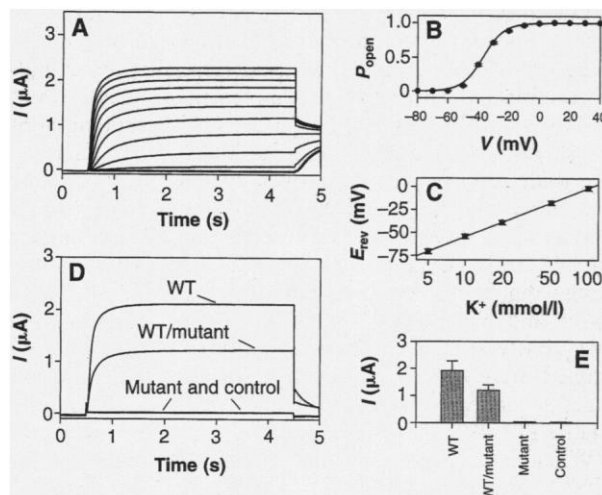


Fig. 2. (A) Segregation of the mutated allele in the Australian pedigree with BFNC. Since the first description of the family (15), individual IV-14 has been newly diagnosed to have febrile convulsions. He is homozygous for the wild-type allele, implying that febrile convulsions are not caused by the *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 ($\sim 80\%$) 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 ($n = 12$, $\pm$ 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: $P_K/P_{\text{Rb}} = 1.27 \pm 0.01$; $P_K/P_{\text{Cs}} = 7.4 \pm 0.5$; and $P_K/P_{\text{Na}} = 51 \pm 4$ ($\pm$ 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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- 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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- Human Northern blot membranes (Clontech) were hybridized with a ³²P-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).
- We thank J. Stoodt and P. Hausmann for excellent technical assistance, G. A. Nicholson and M. L. Kenneron 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. (St769/1-1; 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.

3 December 1997; accepted 15 December 1997