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 posttetanatory potentials (EPSPs) were evoked by stimulating close to the CA1 pyramidial cell layer, and neurons were recorded in bridge mode with sharp microelectrodes containing 2 M potassium acetate (pH 7.3; impedance of 50 to 60 MΩ).

5. Depending on the experiment, the microelectrodes contained 5 mM NEM, 1 mM N19 peptide, 1 mM CaCl₂, 2 mM adenosine triphosphate (Mg²⁺-free), 10 mM Hepes, 10 mM cesium-EGTA, 8 mM NaCl, 1 mM CaCl₂, 2 mM adenosine triphosphate (Mg²⁺-salts), 0.3 mM guanosine triphosphate, 0.2 mM adenosine triphosphate, 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 pulse given at a frequency of 0.1 Hz and were typically in the range of 20 to 150 and 200 to 400 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 dialyzed 1/26 with the patch pipette solution to give a final concentration for SNAP of 80 μM, then briefly sonicated and placed in the tips of the whole-cell pipettes. The pipettes were then backfilled with the standard whole-cell patch pipette solution. Baseline values of EPSCs were obtained from averages of responses during the first 3 min (time 0 on the graph) and defined as 100% for subsequent analyses.


8. The amino acid sequence for the N19 peptide which mimics the NH₂-terminal domain of α- and β-SNAP (designated to disrupt the protein-protein interaction of SNAPs) was GSIFSGLFGSSEKKEACE (26). The peptide was applied on the COOH-terminal and purified by reverse phase high-pressure liquid chromatography.

9. The scrambled S19 peptide, which was prepared in the same way as N19, was GFIESLSFGSEIKEGSF-SCG (26).


11. The botulinum toxin was heated to 90°C for 30 min, and its solubility after denaturation was checked by spectrophotometry.


15. E. Ziff et al., personal communication.

16. J. M. Henley et al., personal communication.


21. It should be noted that while some reduction in the potentiation was evident shortly after the tetanus in the presence of inhibitors of membrane fusion, this potentiation decayed over a 10- to 30-min period. There are a number of possible explanations for this remaining short-term potentiation (STP), which is of longer duration than posttetanic potentiation. One possibility, which we do not favor, is that this STP is mechanistically distinct from LTP. Alternatively, the blockade of membrane fusion may not be complete, and multiple fusion events may be required to stabilize the potentiation.


26. 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.

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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).

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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 (an 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).

(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 KVLT1 sequence. Thin vertical lines indicate the presence of introns in KCNQ2 (reported here) or in KVLT1 (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 (F) in the same region truncates KvLQT1 a few residues downstream in a pedigree with the Jervell and Lange-Nielsen syndrome (24).

(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 cojected 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](image)
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_Ca = 7.4 ± 0.5; and P_K/P_Na = 51 ± 4 (SEM; n = 9). (D) Current traces of WT KCNQ2, a 1:1 conjection 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 mean error bars indicate SEM (n = 5 to 10).

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.

REFERENCES AND NOTES

5. O. Steinlein et al., Genomics 22, 493 (1994).
6. A human fetal brain cDNA library (Clontech) was screened with the insert of cosmid C19-2 (1), 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 PTLa (26). The cDNA sequence of KCNQ2 has been deposited in the European Molecular Biology Laboratory–GenBank database (accession number Y15065).
9. KVLQT1 will be renamed to KCNQ1 by the HUGO/GDB Nomenclature Committee.
13. We obtained partial genomic structure of the KCNQ2 gene by amplifying genomic DNA with gene-specific primers and sequencing the resulting polymerase chain reaction (PCR) fragments with an ABI 377 DNA sequencer. We were guided by the partial genomic structure of KVLQT1 (26), which is largely conserved in KCNQ2 (Fig. 1B). We then designed intronic primers to amplify single exons from genomic DNA. The primers used to amplify the mutant exon in the BFNC pedigree are 5'-GCACAGACAGGCGAACTGGCATGC-3' (forward) and 5'-CTGACCTCCACATGGCCACAG-3' (reverse).
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 (SDS) and of the nucleotide analysis as described (32).
16. The expression vector PTLa (28), containing wild-type or mutant KCNQ2 or ISK (19), was linearized with NotI. Cloned cRNA was transcribed using the Sp6 RNA polymerase in the MessageMachine 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 cDNA was injected into 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 96 mM NaCl, 2 mM KCl, 2.6 mM MgCl2, 0.2 mM CaCl2, and 5 mM HEPES (pH 7.4). For ion substitution experiments, NaCl and KCl were partially substituted by equivalent amounts of KCI (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.
18. M. C. Sanguineti et al., ibid., p. 80.
22. C. Ruibo et al., ibid., p. 267.
23. B. C. Schroeder, C. Kubitsch, T. J. Jentsch, unpublished observations.
32. 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.
33. Human Northern blot membranes (Clontech) were hybridized with a 5'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 film (Kodak XR).
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