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# A novel potassium channel gene, *KCNQ2*, is mutated in an inherited epilepsy of newborns

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**Idiopathic generalized epilepsies account for about 40% of epilepsy up to age 40 and commonly have a genetic basis. One type is benign familial neonatal convulsions (BFNC), a dominantly inherited disorder of newborns. We have identified a sub-microscopic deletion of chromosome 20q13.3 that co-segregates with seizures in a BFNC family. Characterization of cDNAs spanning the deleted region identified one encoding a novel voltage-gated potassium channel, KCNQ2, which belongs to a new KQT-like class of potassium channels. Five other BFNC probands were shown to have *KCNQ2* mutations, including two transmembrane missense mutations, two frameshifts and one splice-site mutation. This finding in BFNC provides additional evidence that defects in potassium channels are involved in the mammalian epilepsy phenotype.**

Benign familial neonatal convulsions (BFNC; OMIM 121200) are an autosomal-dominantly inherited epilepsy of the newborn. This idiopathic, generalized epilepsy typically has an onset of seizures on days 2–4 of life. Spontaneous remission of the seizures occurs between two and fifteen weeks[1–3]. Seizures typically start with a tonic posture, ocular symptoms and other autonomic features, which then often progress to clonic movements and motor automatisms. These neonates thrive normally between the seizures, and their neurological examinations and later development indicate normal brain function[1–3]. Despite normal neurological development, however, seizures recur later in life in approximately 16% of BFNC cases, compared with a 2% cumulative lifetime risk of epilepsy in the general population[1–3].

Linkage analysis in a large kindred demonstrated that a gene responsible for BFNC maps to chromosome 20q13.3, close to the markers *D20S20* and *D20S19* (ref. 4). Soon after the initial report, two centres confirmed linkage of BFNC to the same two genetic markers on chromosome 20, termed the *EBN1* (epilepsy benign neonatal type 1) locus[5–7]. A more distal marker, *D20S24*, shows complete co-segregation with the BFNC phenotype in chromosome-20–linked families. Finding a distal flanking marker for the BFNC locus has not been successful, probably because of its proximity to the telomere. This telomeric region is characterized by a high recombination rate between markers when compared with the physical distance[7]. In fact, Steinlein *et al.* have demonstrated that the three markers *D20S19*, *D20S20* and *D20S24* are contained on the same 450-Mb *Mlu*I restriction fragment[7].

A second chromosomal locus, *EBN2*, has also been identified for BFNC. Lewis *et al.*[8] demonstrated linkage to markers on chromosome 8q24 in a single Mexican-American family affected with BFNC. Evidence for this second locus was also reported in a Northern European pedigree[9]. All of the families in the present study show linkage to chromosome-20q markers with lod scores of greater than 3.0 or have probands with clinical manifestations consistent with BFNC[10]. To find the gene responsible for BFNC, we narrowed the BFNC region with a sub-microscopic deletion in a single family, identified candidate cDNAs in this deletion and then searched for mutations in other BFNC families.

## Deletion of a critical region in a BFNC family

Evidence for a small deletion first came from a genotypic observation with a three-allele RFLP marker, *D20S24*. Analysis of one family, kindred 1547, revealed that a null allele occurred exclusively in individuals with BFNC and in two individuals previously shown to be non-penetrant with the VNTR markers *D20S20* and *D20S19* (Fig. 1). The existence of a deletion co-segregating with the BFNC phenotype in this family was confirmed by fluorescence *in situ* hybridization (FISH) in cell lines of kindred 1547 individuals using as probes the *D20S24* plasmid and two genomic P1 clones containing this marker. The FISH analysis was performed on a single affected individual from K1547 (III-10), a kindred in which affected family members have seizures starting on day 3 or 4. The individual III-10 presented with seizures beginning at three days, and 118 generalized seizures were observed until the age of 23 days. A single seizure was observed at three and a half months in conjunction with an acute infection of the middle ear and fever. No seizures were observed in this individual thereafter. The FISH analysis was car-

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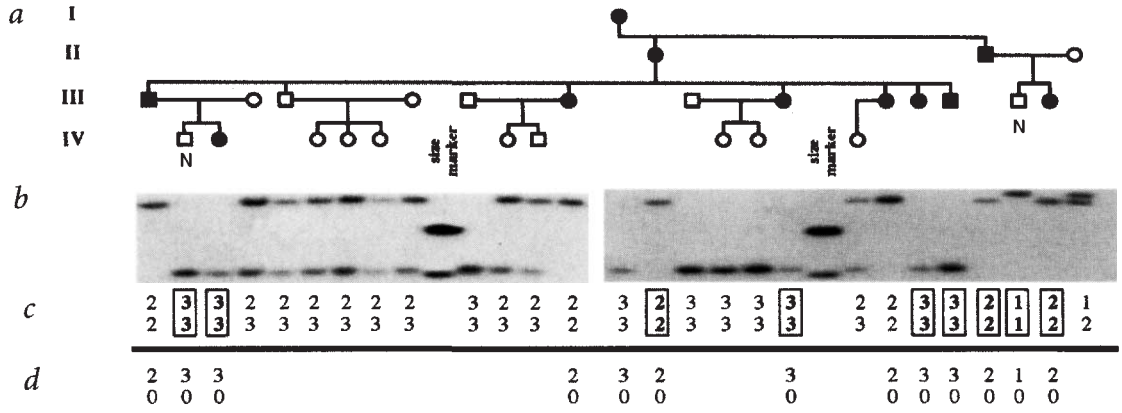


**Fig. 1** A deletion was inferred from a null *D20S24* allele in affected individuals. **a**, Kindred 1547: filled symbols represent affected individuals; N, non-penetrant individuals. **b**, Southern blot of kindred 1547 genomic DNA digested with *TaqI* and probed with *D20S24*; the size marker bands are 4.3 kb and 3.2 kb. **c**, Genotypes with misinheritances shown in boxes. **d**, Corrected genotypes.

ried out blinded with respect to affection status. The analyst examined 36 metaphase spreads, and in the 28 in which a signal was detected, only one chromosome-20 homologue showed hybridization to the *D20S24* plasmid. The 12-kb probe thus gave a hybridization efficiency of 78%. 93% of chromosome spreads of normal individuals exhibited labelling of both chromosomes using the 12 kb *D20S24* probe. The deleted chromosome-20 homologue was identified by G-banding of the chromosomes. These FISH data confirm the existence of a putative null allele shown (Fig. 1) to co-segregate with all affected individuals in the pedigree. Although the 12-kb *D20S24* probe was deleted on one chromosome in affected individuals, the overlapping P1 clones (80 kb in size), which together span approximately 130 kb, showed a positive FISH signal, indicating that the deletion is smaller than 130 kb (Fig. 2).

## Identification of cDNAs in the deleted region

Using the same probes, we identified cDNAs in the region of the deletion by screening a fetal brain cDNA library. A single cDNA isolated with the *D20S24* probe showed significant homology with *KVQT1* (*KCNQ1*), the chromosome-11 potassium-channel gene responsible for the long-QT syndrome and the Jervell and Lange-Nielsen cardio-auditory syndrome[11–13]. Homology extended from amino acid 511 to 562 of *KCNQ1*. A second probe of the fetal brain cDNA library with P1-KO9-6b resulted in the isolation of two additional cDNAs, which showed significant homology with *KCNQ1* amino acids 398–406 and 354–378, respectively. Additional sequence encoding this gene, named *KCNQ2*, was obtained from RACE experiments with adaptor-ligated double-stranded cDNA from fetal and adult brain tissue and from other cDNA clones isolated from a temporal cortex cDNA library.

Composite clones encoding 872 amino acids of *KCNQ2* have been isolated (Fig. 3). The putative initiator methionine lies within a region similar to the Kozak consensus sequence[14]. *KCNQ2* encodes a highly conserved six-trans-membrane motif as well as a pore region, hallmarks of a $K^+$ ion channel gene. The S2, S3 and S4 trans-membrane regions also contain charged amino acids that are found in all members of the $K^+$ channel sub-families, including *Shaker, Shab, Shaw* and *Shal*. A search of GenBank with *KCNQ2* sequence shows identical nucleotide sequence to *HNSPC*, a 393-amino-acid putative potassium-channel cDNA isolated from a human neuroblastoma cell line[15]. However, the last 21 amino acids of *HNSPC*, including a stop codon, are encoded by a sequence that in *KCNQ2* is intronic. A search of the human expressed-sequence-tag database (dBest) shows seven clones encoding portions of *KCNQ2*. Wei *et al.* have identified a gene from *Caenorhabditis elegans*, *nKQT1*, that appears to be a homo-

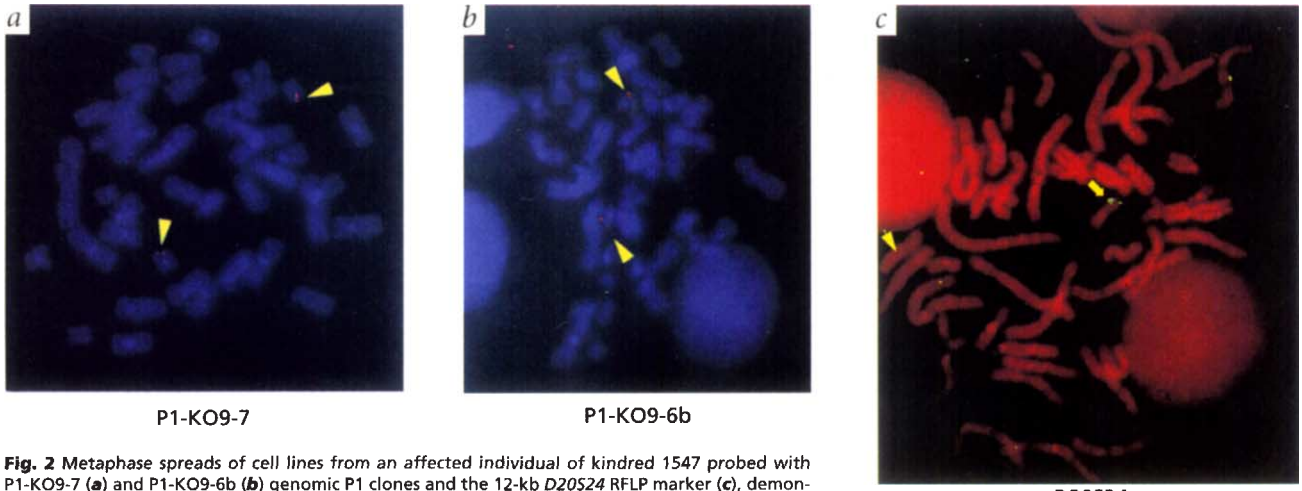


**Fig. 2** Metaphase spreads of cell lines from an affected individual of kindred 1547 probed with P1-KO9-7 (**a**) and P1-KO9-6b (**b**) genomic P1 clones and the 12-kb *D20S24* RFLP marker (**c**), demonstrating a deletion of *D20S24*.

**Table 1 • Mutations in *KCNQ2* in BFNC families**

| Mutation | Region | Kindred | Controls | Nucleotide change |
|---|---|---|---|---|
| deletion | n/a | K1547 | 70 | n/a |
| 283insGT | pore | K1504 | 70 | frameshift |
| Y284C | pore | K3904 | 70 | TAC to TGC |
| A306T | S6 | K1705 | 70 | GCG to ACG |
| 522del 13 | C-terminal | K3369 | 70 | frameshift |
| 544-1G to A | C-terminal | K3933 | 70 | splice-site variant |

n/a, not available.

logue of *KCNQ2* (ref. 16). This group also described the human EST homologue of *nKQT1*, *hKQT2*, which is a partial clone of *KCNQ2* (ref. 16). In addition to the six trans-membrane domains and the pore, a small region 5′ of trans-membrane domain S1 is also conserved between *KCNQ2*, *KCNQ1* and *nKQT1*. Unlike other $K^+$ channel sub-families, the C-terminal domain appears to contain highly conserved residues for *KCNQ2*, *nKQT1* and *KCNQ1* (Fig. 3). 3′–RACE has not led to the identification of the poly-A tail or the polyadenylation signal, although 486 nucleotides have been obtained after the termination codon.

### *KCNQ2* expression and mutations in BFNC patients

The *KCNQ2* cDNA hybridizes to transcripts approximately 1.5, 3.8 and 9.5 kb in size on northern blots made from brain (data not shown). The 1.5- and 9.5-kb transcripts appear to be expressed in both adult and fetal brain. The 3.8-kb transcript is expressed in select areas from adult brain, particularly in the temporal lobe and the putamen.

Mutational analysis of *KCNQ2* was performed on one affected individual from each of our twelve BFNC families or unrelated probands. In addition to the substantial deletion in kindred 1547, mutations were identified in five other BFNC families. Mutational analysis was carried out by first screening probands for SSCP variants and then sequencing each proband's DNA to determine the basis for the molecular variation. Mutations identified include two missense mutations, two frameshift mutations and one splice-site mutation (Table 1). None of the mutations identified were seen in SSCP analysis of our panel of 70 unrelated, unaffected individuals. In our collection of twelve BFNC probands, three (K1504, K1547, K1705) were from families that showed significant linkage, with lod scores greater than 3.0 on chromosome 20 (ref. 10). Mutations were found in all three families. Two were single probands, and a splice-site mutation was found in one (K3933). The remaining seven families were too small for linkage to chromosome 20 to be demonstrated. In this group, mutations in *KCNQ2* were observed in two families (K3904 and K3369). The complete *KCNQ2* gene has not been screened for mutations in the six remaining pedigrees. Furthermore, mutations were shown to segregate completely with affection status in all of the BFNC families in which mutations were identified. An example of this segregation is shown in Fig. 4 for the 2-bp insertion identified in kindred 1504; all eleven affected members have the SSCP variant, and all seven unaffected individuals have wild-type SSCP bands. Two neutral polymorphisms were identified in our 70 control individuals (CEPH parents)—one in codon 304 (TTC→TTT) in the S6 trans-membrane domain, with a frequency of 7.0%, and a second in codon 573 (GCC→GCT) in the 3′ region of *KCNQ2*, with a frequency of 0.58%.

It is predicted that the splice-site mutation in the conserved 3′ region of *KCNQ2* and the two frameshift mutations, one in the pore region and one before the highly conserved 3′ region, lead to altered protein products. In the case of the 283insGT pore mutation, a predicted stop codon is found 36 amino acids downstream; in the case of the 522del13 3′ mutation, a predicted stop codon is found two amino acids downstream. Also, the 2-bp insertion mutation, 283insGT, would lead to a completely altered S6 trans-

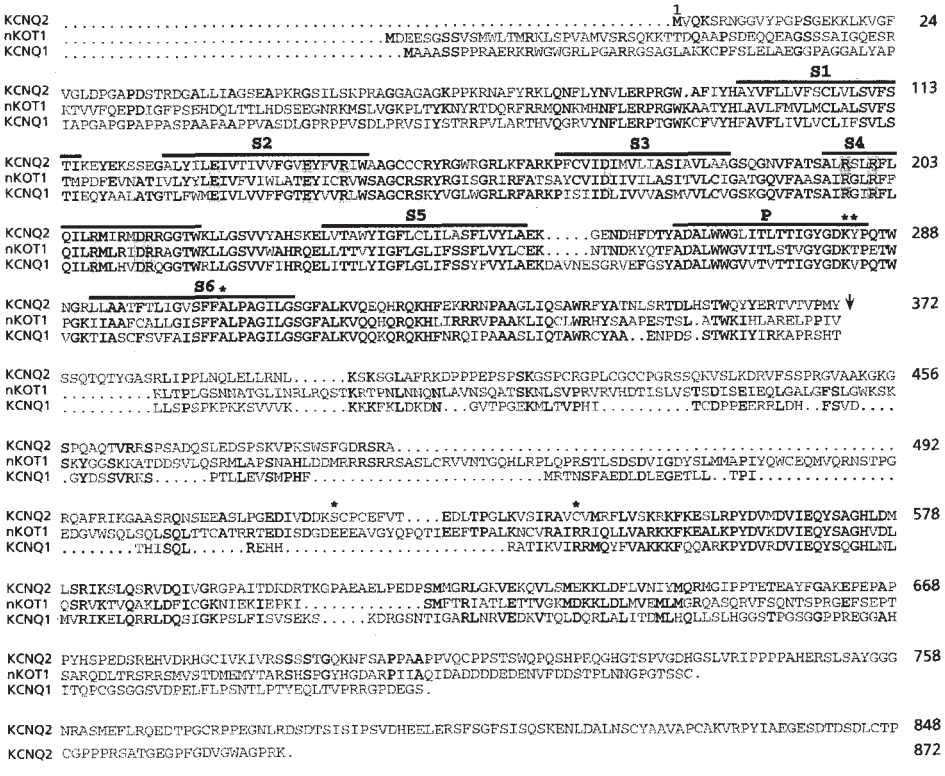


**Fig. 3** Amino-acid alignment of KCNQ2 with KCNQ1 and nKQT1 from *C. elegans*. The arrow indicates the exon–intron boundary that is read through in the *HNSPC* clone; an asterisk indicates the sites of mutations listed in Table 1. KCNQ2 and KCNQ1 share 60% identity and 70% similarity in the trans-membrane regions from S1 to S6. Grey shading indicates conserved charged amino acid residues in S2, S3 and S4.

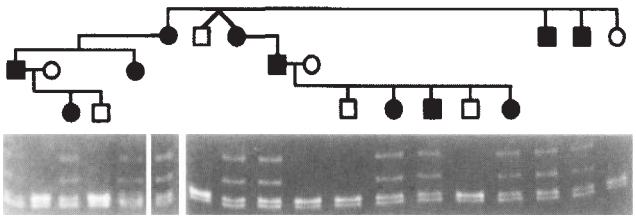

**Fig. 4** Segregation of SSCP variants in the KCNQ2 pore region in kindred 1504. Sequence analysis revealed the existence of a 2-bp insertion in affected individuals showing the upper two (variant) bands.

membrane domain. Although the breakpoints of the kindred-1547 deletion have not been determined, it is known that the 12-kb plasmid, which includes the RFLP marker locus, *D20S24*, contains at least 80 codons (residues 509–588 of *KCNQ2*) of sequence from the highly conserved 3′ region of the *KCNQ2* gene, indicating that at least this portion of the gene is deleted in affected kindred-1547 individuals. The deletion in this family may also include other cDNAs. The two missense mutations change amino-acid residues in key functional domains, the pore and S6 domains. Six unique mutations have been identified in *KCNQ2* to date. The mutation defined by a 13-bp deletion at amino-acid 522 in kindred 3369 is of interest in that there is a greater variation in the reported clinical ages at onset within this family than in typical BFNC families. In kindred 3369, three individuals had onset of seizures within the first two weeks of life, whereas three individuals had onset of seizures at three, four and five months of age.

## Discussion

This is the first human idiopathic generalized epilepsy in which a $K^+$ channel has been implicated. BFNC is considered to be a true idiopathic epilepsy because seizures are the only manifestation observed. This is in contrast to other epileptic syndromes, which have degenerative characteristics and an epileptic component, such as progressive myoclonus epilepsy of the Unverricht-Lundborg type. It is not surprising, therefore, that an alteration in a gene that directly regulates neuronal excitability could produce the epileptic disorder seen in BFNC. Voltage-gated potassium channels repolarize neuronal membranes that have been depolarized by $Na^+$ and $Ca^{++}$ voltage-gated ion channels. $K^+$ channels are also thought to repolarize neuronal membranes after activation of excitatory neurotransmitter ion channels, including glutamate and acetylcholine. In the presence of mutant KCNQ2 channels with reduced function, excitatory ligand and voltage-gated channels that are activated would remain open longer[17–19]. Such unchecked activity of excitatory systems could lead to an epileptic phenotype. Additional studies using brain slices and whole animal models have implicated altered $K^+$ regulation as having a causal role in epilepsy[19]. Electrophysiological analysis of the mutant KCNQ2 channels will shed light on how the mutations identified in the current study produce an epileptic phenotype. It is likely that KCNQ2 will have biophysical properties similar to the delayed rectifier KCNQ1 channel. KCNQ1 alpha subunits co-assemble with minK beta subunits to form heteromultimeric $I_{Ks}$ channels in the heart[20]. It is possible that KCNQ2 subunits co-assemble with minK-like beta subunits in the brain. This interaction may also alter the gating properties of the resulting heteromultimeric channel, as is the case for KCNQ1.

Mutations in $K^+$ channels have been associated with epilepsy in only one other case—the weaver mouse, in which a single missense mutation in the *GIRK2* gene produces spontaneous seizures[21,22]. Mutations in $K^+$ channels have been implicated in other human disorders, such as the long-QT syndrome on chromosome 11 and ataxia/myokymia on chromosome 12 (refs 11,13,23–25). Long-QT syndrome is associated with four loci, two of which are the $K^+$ channel genes *HERG* and *KCNQ1*. In *KCNQ1*, mutational hot spots have been identified in the pore and S6 domains, where missense mutations account for most of the disease-causing mutations in LQT[11,23].

The mutation in the BFNC kindred 1705 is an alanine→threonine substitution in the S6 trans-membrane segment. This alanine residue is conserved in all members of the *Shaker, Shab, Shaw* and *Shal* sub-families of potassium channels identified to date[26,27]. The *KCNQ1* gene, to which the *KCNQ2* ion channel is most closely related, also contains an alanine in this position. In six unrelated LQT1 families, the disease-causing mutation occurs at the same position, where the alanine is changed to a valine[11,23]. This S6 trans-membrane domain has been shown to be involved in $K^+$ ion permeation in the *Shaker* subtype[28], and it may serve a similar function in *KCNQ2*. The C-terminal region appears to be important for gene function because a 13-bp deletion and a splice-site mutation both produce an epileptic phenotype in separate BFNC families (Table 1, Fig. 3). The same region is known to have a deletion–insertion mutation in *KCNQ1* in individuals with the Jervell and Lange-Nielsen recessive form of LQT and associated deafness[13]. Disease-causing mutations in the C-terminal region further argue for a functional protein encoded by the *KCNQ2* gene rather than the shorter *HNSPC* clone.

The pore region of $K^+$ ion channels belonging to the same structural class have been characterized extensively by mutational analysis[29]. The 2-bp insertion observed in kindred 1504 occurs immediately after the universally conserved GYG motif. An insertion here not only alters the length of the pore that is believed to be crucial for function[29–32] but also modifies the signature sequence of the pore and produces a truncated protein.

In infants of families that have been linked to the chromosome-20 form of BFNC, EEG recordings show initial suppression of activity throughout the brain, followed by generalized discharges of spikes and slow waves[1–3]. In adults, the *KCNQ2* gene is expressed in various parts of the brain. Cortical regions as well as sub-cortical areas, such as the thalamus and caudate nucleus, contain transcripts of *KCNQ2* of various sizes (data not shown). It is possible that this expression pattern is also the same in the newborn infant.

The close homology (60% identity and 70% similarity of amino acids in the trans-membrane region from S1 to S6) of *KCNQ2* with *KCNQ1* and with the *C. elegans nKQT1* gene and the reduced homology of these channels with the *Shaker, Shab, Shaw* and *Shal* sub-families imply that they belong to a unique family of $K^+$ ion channels, called KQT-like by Wei *et al.*[16]. A new $K^+$ ion channel now known to be expressed in the brain raises the question of whether additional, undiscovered members of this gene family might be responsible for other forms of idiopathic, generalized epilepsies with tonic-clonic convulsions. A similar idiopathic seizure disorder seen early in development is benign familial infantile convulsions (BFIC). In BFIC, the seizures begin at four to eight months of age and remit after several years. BFIC maps to chromosome 19q in five Italian families[33,34]. It is reasonable to hypothesize that BFIC and the chromosome-8 form of BFNC are also caused by mutations in as yet unidentified members of the KQT-like family of $K^+$ ion channels or by minK-like proteins.

In conclusion, we have shown that a genetic defect of a potassium channel is associated with the human idiopathic epilepsy of BFNC. This finding adds to the growing list of channelopathies in humans[17,35,36] and suggests that drugs that directly or indirectly modulate $K^+$ ion channels will be helpful in the treatment of seizure disorders.

## Methods

**Southern-blot analysis.** Genomic DNA (5 µg) was cut with *TaqI* and transferred to a nylon membrane. Filters were hybridized overnight at 65 °C in PEG hyb (7% PEG, 10% SDS, 50 mM $NaPO_4$ and 200 µg/ml total human DNA) with the *D20S24* plasmid probe labelled by random priming (Stratagene). Filters were washed at 2× SSC, 0.1% SDS twice at room temperature, followed by one wash in 0.5 × SSC, 0.1% SDS at 65 °C.

**Fluorescence *in situ* hybridization.** Chromosomes from transformed lymphocytes were prepared with a 30-min ethidium-bromide treatment, followed by 3 h in Colcemid (CIBA). Cells were then pelletted and re-suspended in hypotonic solution (0.75 M KCl) for 20 min, followed by the addition of four or five drops of fresh fixative (3:1 methanol:acetic acid). Cells were again pelletted, vortexed and then carefully re-suspended in fixative. After three washes in fixative, metaphases were stored at 4 °C. Probe (400 ng) was labelled with biotin and hybridized to slides of metaphase spreads by means of standard hybridization procedures. Probes were then fluorescently tagged with avidin-FITC (Vector); the signal was intensified with biotin-labelled anti-avidin, followed by avidin-FITC. The chromosomes were then counterstained with DAPI and visualized with a Zeiss Axioplan fluorescent microscope equipped with FITC, DAPI and triple-band-pass filter sets. Images were captured with Probevision software (Applied Imaging) and photographs printed on a Kodak XL 7700 colour image printer.

**Full-length cDNA.** A total of $10^6$ clones of a fetal brain library (Stratagene) were probed sequentially with inserts from P1-KO9-6b and P1-KO9-7, and the plasmid *D20S24* was labelled by random priming (Stratagene) with $^{32}$P-dCTP (Du Pont–NEN). Hybridizations were performed in 5× SSC, 10× Denhardt's, 0.1 M $NaPO_4$ (pH 6.7), 100 µg/ml salmon sperm DNA, 0.1% SDS and 50% formamide. Blots were washed in 2× SSC, 0.1% SDS twice at room temperature, followed by one wash in 0.5× SSC, 0.1% SDS at 42 °C. To identify the full-length gene, 5′– and 3′–RACE were performed on adaptor-ligated fetal and adult brain cDNA (Clontech); PCR products were sub-cloned (Invitrogen) and sequenced on an ABI 377 (Advanced Biotechnologies). Additional sequence came from screening a temporal cortex cDNA library (Stratagene).

**Mutational analysis.** Coding regions S1–S6 and conserved regions in the 3′ end of *KCNQ2* were amplified by PCR with primers within introns and analysed by SSCP with 20% polyacrylamide gels containing TBE buffer (Novex) run at 4 °C. The exon–intron boundaries were identified by sequencing products obtained by exon–exon PCR on genomic P1 clones or directly from RACE products that contained unprocessed transcripts. PCR products showing variants seen on SSCP were either cloned and sequenced or re-amplified with M13 reverse and M13 universal-tailed primers and sequenced directly on an ABI 373 or 377 (Advanced Biotechnologies) with dye-primer chemistry.

**GenBank accession numbers.** The number for *KCNQ2* is AF033348; that for *HNSPC* is D82346.


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