A Reduced K^+ Current due to a Novel Mutation in KCNQ2 Causes Neonatal Convulsions

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Benign familial neonatal convulsions (BFNC) is a rare dominantly inherited epileptic syndrome characterized by frequent brief seizures within the first days of life. The disease is caused by mutations in one of two recently identified voltage-gated potassium channel genes, KCNQ2 or KCNQ3. Here, we describe a four-generation BFNC family carrying a novel mutation within the distal, unconserved C-terminal domain of KCNQ2, a 1-bp deletion, 2513delG, in codon 838 predicting substitution of the last seven and extension by another 56 amino acids. Three family members suffering from febrile but not from neonatal convulsions do not carry the mutation, confirming that febrile convulsions and BFNC are of different pathogenesis. Functional expression of the mutant channel in *Xenopus* oocytes revealed a reduction of the potassium current to 5% of the wild-type current, but the voltage sensitivity and kinetics were not significantly changed. To find out whether the loss of the last seven amino acids or the C-terminal extension because of 2513delG causes the phenotype, a second, artificial mutation was constructed yielding a stop codon at position 838. This truncation increased the potassium current by twofold compared with the wild type, indicating that the pathological extension produces the phenotype, and suggesting an important role of the distal, unconserved C-terminal domain of this channel. Our results indicate that BFNC is caused by a decreased potassium current impairing repolarization of the neuronal cell membrane, which results in hyperexcitability of the central nervous system.

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Benign familial neonatal convulsions (BFNC) is one of the rare idiopathic epileptic syndromes with a dominant mode of inheritance. The penetrance of BFNC is as high as 85%. Affected individuals typically suffer from frequent brief seizures that occur within the first days of life and disappear spontaneously after weeks to months. The neurological examination, interictal electroencephalogram (EEG), and development of these children are usually normal. The risk of recurring seizures later in life is about 15%.[1–3] In 1989, the disease was mapped to the long arm of chromosome 20.[4] A second locus on chromosome 8, suggesting genetic heterogeneity, has been described.[5] Recently, mutations in two novel voltage-gated potassium channel genes, KCNQ2 (20q13.3) and KCNQ3 (8q24), have been identified.[6–8]

Ion channels provide the basis for the regulation of excitability in the central nervous system and other excitable tissues such as skeletal and heart muscle. It is therefore not surprising that mutations in channel-encoding genes, leading to a dysfunction of these highly specific, membrane-spanning proteins, result in hyperexcitability or hypoexcitability of the corresponding cells. In the past 10 to 15 years, the combination of electrophysiological and molecular genetic investigations led to the identification of the growing family of the so-called channelopathies. Examples for known ion-channel disorders are the periodic paralyses and nondystrophic myotonias, inherited cardiac arrhythmias (the long-QT syndromes), episodic ataxias, and familial hemiplegic migraine.[9,10] In addition to BFNC, two more inherited human epileptic syndromes have so far been linked to mutated ion channels. In autosomal dominant nocturnal frontal lobe epilepsy, mutations within the α-subunit of a neuronal nicotinic acetylcholine receptor have been reported[11,12] and a mutation in

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a sodium channel β-subunit is associated with a dominantly inherited epileptic syndrome including febrile and generalized seizures.[13]

These genetically determined diseases provide an excellent tool to study pathophysiology in molecular detail, and such studies may serve as a model for hyperexcitability and epilepsy in general. To prove that the mutations are really causative for the reported diseases, the first step involves expression and functional characterization of the mutant channels in comparison with the wild type (WT), to look for significant differences in channel behavior that can explain the pathogenesis. Whereas such studies have been performed extensively for the peripheral channelopathies,[9] they are rare for the mentioned epileptic syndromes. First reports include an accelerated desensitization and a reduced single-channel conductance for mutations in the acetylcholine receptor α_4-subunit, producing a hypoactive channel.[12,14,15] For BFNC it has been reported that a mutation in KCNQ2 truncating the last 300 amino acids leads to a nonfunctional channel protein when expressed in *Xenopus* oocytes.[8] This would explain the hyperexcitability via a reduced potassium-dependent repolarization of the neuronal cell membrane similar to mutations in KCNQ1 causing the long-QT syndrome.[16,17] Expression of the mutation in the sodium channel β-subunit yielded a slowing of channel inactivation,[13] which also explains hyperexcitability, as has already been shown for many mutations in the human skeletal or heart muscle sodium channel α-subunits causing myotonia or long-QT syndrome type 3.[9,10]

Here, we describe a large, four-generation Sicilian family with BFNC, the identification of a novel mutation in the distal, unconserved C-terminal domain of KCNQ2, and functional expression of the mutant channels in *Xenopus* oocytes. The correlation of clinical, genetic, and electrophysiological data allows us to determine the molecular pathophysiology of this benign epileptic syndrome in our family.

Materials and Methods

EDTA blood probes were taken with informed consent from all affected and nonaffected individuals available. Genomic DNA was extracted by a standard procedure.

Single-Strand Conformational Analysis and Heteroduplex Analysis

Polymerase chain reaction (PCR) amplification was performed for 33 cycles at 94°C for 30 seconds, 65°C for 30 seconds, and 72°C for 30 seconds after an initial denaturation step at 94°C for 5 minutes. The mutation-carrying fragment in exon 16 was amplified, using primers forward, 5′GTGGACCACGAGGAGCTGGA, and reverse, 5′AACCTCGGAGGCACCGTGCT. For single-strand conformational analysis, 5 μl of the amplified product was mixed with 7 μl of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% bromphenyl blue (BPB), and 0.05% xylene cyanol). The samples were denatured for 5 minutes at 95°C and then 7 μl was loaded onto 10% polyacrylamide gels (37.5:1 acrylamide:bis, 10% [vol/vol] glycerol, 0.5 × TTE). Samples were run for 14 to 16 hours at 5 V/cm at room temperature and 4°C in 0.5 × TTE running buffer. After the run, the gels were silver stained.[18]

For heteroduplex analysis, 2 μl of the PCR product was denatured (95°C, 5 minutes) and reannealed (50°C, 30 minutes), and mixed with 5 μl of BPB loading buffer (with 8% Ficoll 400, 20 mM EDTA, 0.05% BPB, 0.05% xylene cyanol). Reannealed products were separated on 10% polyacrylamide gels at 18 V/cm at room temperature for 2 hours and then silver stained. Products showing polymorphisms were directly sequenced using an ABI 377 sequencer, and the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA).

Restriction Assay of the Mutation

Part of exon 16 was amplified by using the following primers: forward, 5′GAGGGTCCCTTTGGTGACGTAG, and reverse, 5′AACCTCGGAGGCACCGTGCT. PCR was performed with 5% dimethyl sulfoxide and an annealing temperature of 60°C. The mismatching second base at the 3′ end of the forward primer was introduced to create an artificial *Alu*I restriction site in the mutant but not in the wild-type allele; 5 μl of PCR product was digested to completion, mixed with BPB loading buffer, and analyzed on a 10% polyacrylamide gel. After *Alu*I digestion, the following band pattern was observed: normal allele, 104 bp, mutant allele, 83 bp and 21 bp.

Mutagenesis and RNA Preparation

The 1-bp deletion 2513delG was introduced into the previously described KCNQ2 expression construct[8] by using the Gene Editor in vitro site-directed mutagenesis system (Promega Corporation, Madison, WI) with the following oligonucleotide: 5′GAGGGTCCCTTTGGTGACGTCGCTGGGCCGGGCCCAGGAAGTG. Because the original expression construct did not contain all of the nucleotides coding for the 56 amino acids until the new stop codon of the mutant gene (Fig 1), a 140-bp fragment containing *Sac*II and *Xba*I restriction sites was cloned by PCR from human genomic DNA and inserted into the vector, using the following primers: 5′GTGGACCCGCCCGCGGCCCTC and 5′CGGTACCTCTAGATCACTGCCAGGAGCCCCCATCC. To generate a channel truncated at the site of the deletion, a second mutation was made introducing a stop codon at position 838, using the same mutagenesis system ("G838X," oligonucleotide: 5′GGGTCCCTTTGGTGACGTCTGATGGGCCGGGCCCAGGAAG). All mutations were verified by sequencing. To reproduce the mutant phenotype, at least two clones from the mutagenesis procedures of each mutation were expressed in oocytes. The expression vector containing either WT or mutant cDNA was linearized with *Mlu*I. RNA was prepared by using the SP6 CAP-Scribe Kit from Boehringer (Mannheim, Germany).

Oocyte Preparation and Injection

Pieces of ovary were surgically removed from anesthetized *Xenopus laevis* frogs with a length of at least 9 cm (African

Xenopus Facility, Noordhoek, South Africa). Oocytes were defolliculated by using a 2-hour collagenase treatment (2 mg/ml of type CLS III collagenase; Biochrom KG, Berlin, Germany) in OR-2 solution (in mM: 82.5 NaCl, 2 KCl, 1 mM Mg Cl_2, 5 mM HEPES, pH 7.4). They were stored at 18°C in L-15 Medium Leibovitz (Sigma, Deisenhofen, Germany) supplemented with 50 μg/ml gentamicin (Biochrom KG, Berlin, Germany). Undiluted RNA (70 nl) was injected into each oocyte. Assuming a density of 0.5 to 1 μg/μl RNA, this corresponds to approximately 50 ng of RNA per oocyte. For the determination of the current amplitude/expression level of WT and both mutations, RNA preparations from the WT and the two mutant clones, yielding equal bands on a gel, thus having approximately the same density, were injected on the same day into the same charge of oocytes and measured in parallel on days 2 to 4 after injection. The results shown in Figure 1B were obtained exclusively on day 3 after injection, where current size was maximal in all three clones. The same was done with a 1:1 mixture of WT and each mutant. The results were reproducible with at least two other charges of oocytes in different experiments.

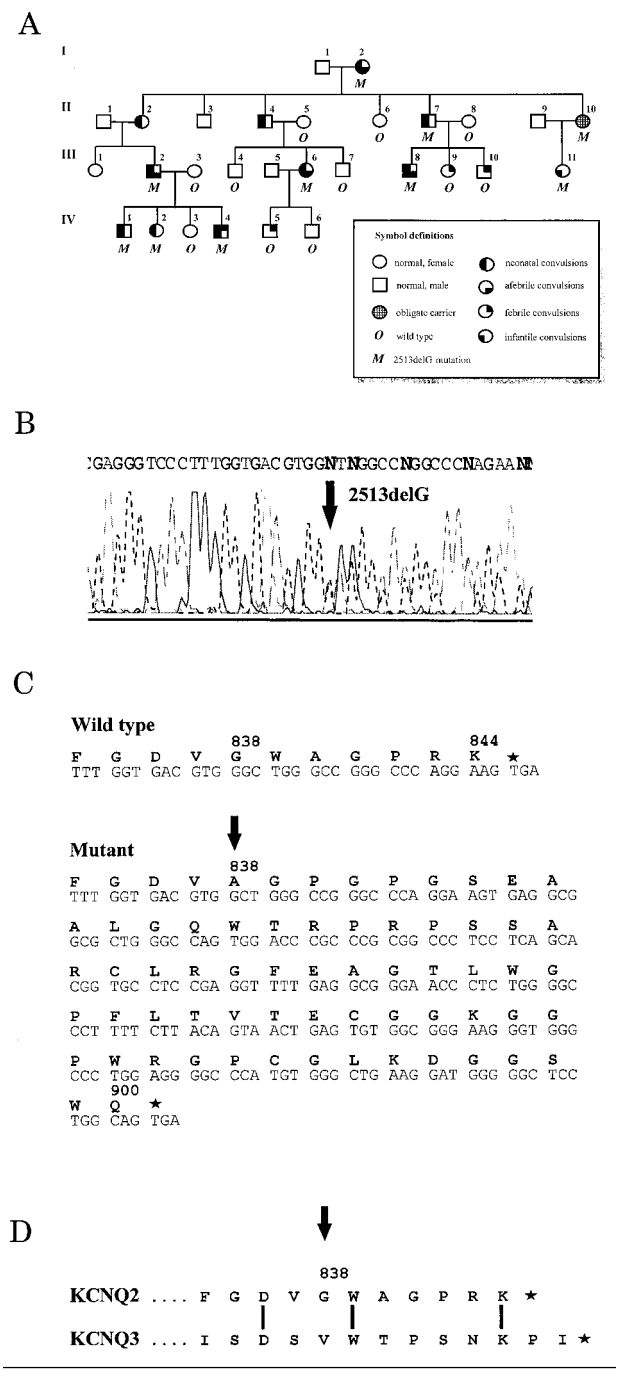


Fig 1. Clinical and genetic data of the four-generation benign familial neonatal convulsions (BFNC) family. (A) Pedigree of the Sicilian BFNC family, showing the clinical and genetic phenotype as indicated in the legend (M = *heterozygous for the 2513delG mutation in exon 16;* 0 = *homozygous for the wild type*). *More detailed clinical information is given in Results. (B) Double-stranded sequencing of Individual III-2. The* arrow *marks the position of the 1-bp deletion. (C) Partial nucleotide and deduced amino acid sequence of the wild-type and mutant KCNQ2 gene. The 1-bp deletion causes a frameshift at codon 838* (*indicated by an* arrow), *subsequently abolishing the stop codon at position 845 and adding 56 codons to the 3′ end. (D) Alignment of the wild-type channel C-terminus between KCNQ2 and KCNQ3. Conserved amino acids are marked by* vertical lines. *The position of the 2513delG mutation is indicated by an* arrow.

Electrophysiology

Potassium currents were measured on day 2 to 5 after injection, using a standard two-microelectrode voltage clamp technique with a Turbo TEC 01C amplifier (npi electronic GmbH, Tamm, Germany) and pClamp 6.02 data acquisition (Axon instruments, Foster City, CA). Electrodes had a resistance of 0.3 to 1 MΩ, when filled with 3 M KCl. The bathing solution (frog Ringer) contained (in mM): 115 NaCl, 2.5 KCl, 1.8 $CaCl_2$, and 10 HEPES, pH 7.4. Injected and uninjected oocytes had resting membrane potentials between −45 and −60 mV. Currents were low-pass filtered at 0.5 and sampled at 1 kHz. Data were analyzed with a combination of pClamp, Excel '97 (Microsoft, Redmond, WA) and Origin 5.0 (Microcal Software, Northampton, MA) software. For statistic evaluation, Student's *t* test was applied. All data are shown as mean ± SEM values.

Results

Clinical Data

The Sicilian pedigree is shown in Figure 1A. Detailed information about the clinical history was available for the index patient (Individual IV-4) and his siblings (Individuals IV-1 and IV-2), who are living in Germany. Data were obtained by history from the parents, as well as from medical records of the Department of Pediatrics of the University of Ulm. The whole index family (Individuals III-2-3 and IV-1-4) showed a normal neurological examination during a visit to Ulm.

The index patient had his first seizure on day 5 after

birth. His mother described a recurring seizure type, starting with apnea, cyanosis, and a diffuse hypertonia followed by clonic movements of the limbs. Seizures lasted about 30 seconds and occurred singularly or in series. He was successfully treated with phenobarbital. After stepwise discontinuation of the medication after 2 months, he again suffered from seizures, lasting now up to 5 to 10 minutes before treatment with diazepam. He was again treated with phenobarbital. At the age of 5 months, he had two more seizures during a febrile gastroenteritis under anticonvulsive treatment. The medication was continued until the age of 18 months. He had no further seizures up to now (at 4 years of age). Several interictal EEGs were normal. A computed tomographic scan at the age of 3 months showed slight asymmetry of the temporal lobes and a small (5 mm diameter) probable arachnoid cyst in the left paramedian parietal lobe. His clinical neurological examination was always normal.

His affected sister (Individual IV-2) suffered from a few mild seizures on days 1 and 2 after birth, starting with oral movements followed by a generalized stiffening that lasted no longer than 10 seconds. She was treated with phenobarbital for 4 days and had no further seizures. For several interictal EEGs, no epileptic discharges were reported. A subsequent computed tomographic scan at the age of 3 weeks showed no abnormalities. Her neurological examination was always normal.

Neonatal seizures of the oldest son of the family (Individual IV-1) occurred first on day 5 of life. They started with an initial shrill cry and hypertonia followed by limb movements and lasted 5 to 10 seconds (3 or 4 seizures per day). He was treated with phenobarbital for 5 months and never had any additional seizures. Thus, all 3 children had an individual recurring seizure type.

Information about the rest of the pedigree was obtained during a visit to Sicily by taking history from many family members and from a general medical doctor of the family. Of the family members who had neonatal convulsions, as indicated in Figure 1A, Individual I-2 has experienced additional, presumably generalized tonic–clonic seizures, with loss of consciousness, hypersalivation, bite of the tongue, and without any signs of an aura, occurring after the age of 60 years. Individual III-2 reported two seizures with loss of consciousness at the age of 18 years without obvious provoking factors. Individual III-6 had seizures until she was 4 years old. Neonatal seizures of Individual III-8 started on day 3 after birth and lasted about 3 weeks. He had two more generalized tonic–clonic seizures between 15 and 16 years of age. Individual III-11 did not suffer from neonatal seizures but experienced frequent, probably generalized tonic–clonic seizures, with cyanosis, hypersalivation, hypertonia, and clonic jerks of the whole body, during 1 week within the sixth month of life. Her mother reported five to 12 seizures per day. Three individuals had febrile convulsions (FCs) at the age of 4 or 5 years but no neonatal seizures, as indicated in Figure 1A.

Molecular Genetic Analysis

Initial analysis of the index family (Individuals III-2-3 and IV-1-4), using the polymorphic markers D20S214 and D20S617, suggested linkage to the known locus on chromosome 20q13.3. Subsequently, single-strand conformational analysis and heteroduplex analysis was used to scan the whole coding region of KCNQ2 in DNA samples derived from the index family. PCR fragments indicative of a mutation were found in exon 16. Subsequent sequencing revealed a 1-bp deletion mutation, 2513delG, in codon 838 located close to the end of the open reading frame (see Fig 1B). The subsequent frameshift would alter the 3′ end of the KCNQ2 gene, resulting in substitution of the last seven amino acids and in an extension of the channel protein by another 56 residues (see Fig 1C). The mutation was not observed in 154 normal population controls. No other mutations were found by screening the whole KCNQ2 coding region in the Sicilian family by single-strand conformational analysis and heteroduplex. Southern blot analysis of the 12-kb D20S24 probe demonstrated Mendelian inheritance of this polymorphism in the Sicilian pedigree, thus excluding the submicroscopic deletion previously found in several other BFNC patients[7] (Steinlein and colleagues, unpublished data).

To examine the segregation of the mutation, heteroduplex and restriction enzyme assays in DNA samples from all individuals available were performed (results are shown in Fig 1A). The 2513delG mutation was found in all affected pedigree members available for testing, including 1 individual (Individual III-11) for whom seizures were reported in the sixth month of life but not in the neonatal period (see above). The mutation was also found in her unaffected mother (Individual II-10), who therefore was considered an obligate carrier. Three individuals (Individuals III-9, III-10, and IV-5) experiencing FCs but no neonatal or other afebrile seizures did not carry the mutation, confirming that BFNC and FCs are of different pathogenesis.[4,8]

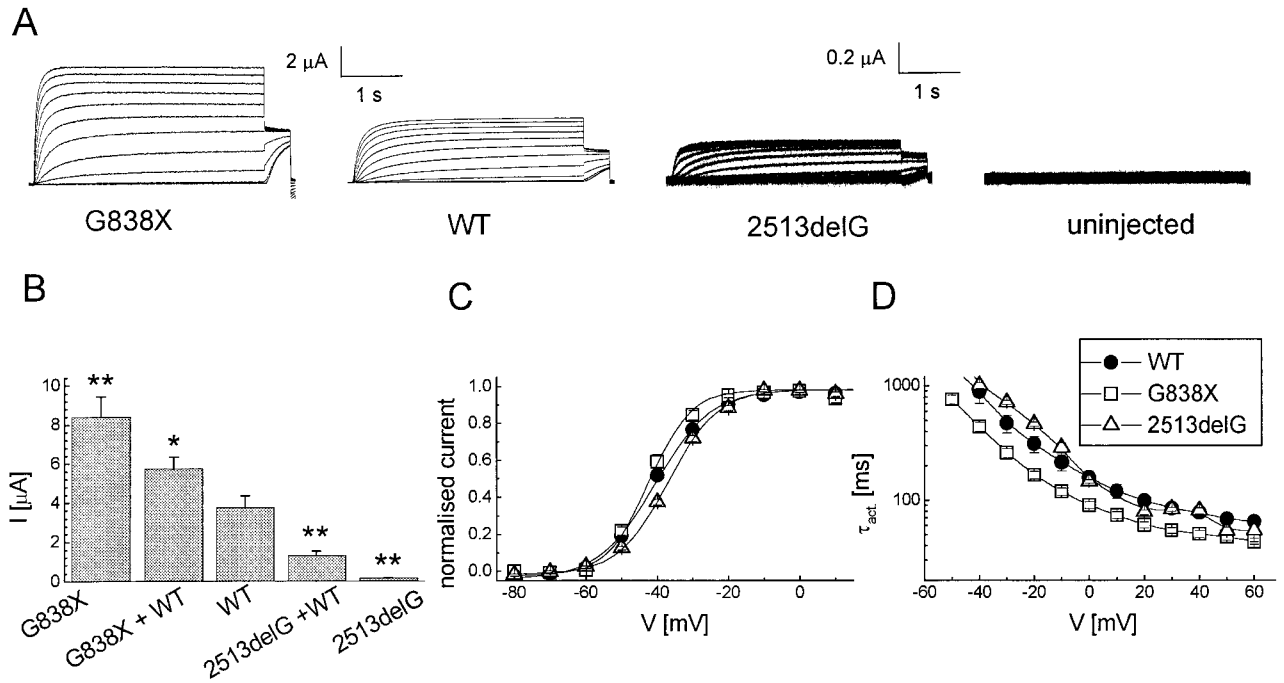
Electrophysiology

The next step was functional expression of the mutation to prove its disease-causing role for BFNC in the reported family. The 1-bp deletion 2513delG was introduced in the cDNA of KCNQ2 by site-directed mutagenesis, and both WT and mutant channels were expressed in *Xenopus laevis* oocytes as described in Materials and Methods. Potassium currents were elicited by depolarizing test pulses from a holding potential of

−80 mV. They activated slowly at −50 mV and more positive potentials. A striking difference between WT and mutant channels was seen in current amplitude; ie, currents derived from 2513delG had, on average, less than 5% of the WT amplitude (0.16 ± 0.01 μA [n = 16] vs 3.8 ± 0.6 μA [n = 13] at the end of a 4-second depolarizing test pulse to +10 mV; Fig 2A and B). Coexpression of WT and mutant channels yielded an average current amplitude of 1.3 ± 0.2 μA (n = 12), not supporting a significant dominant negative effect of the mutation ($p > 0.05$). In a different set of experiments, we compared the current amplitude in oocytes injected with only WT RNA (35 nl) with those oocytes injected with the same amount of WT RNA plus the same amount of mutant RNA (35 nl each). Current amplitudes in those two sets of oocytes were very similar (0.9 ± 0.1 μA for WT vs 1.0 ± 0.1 μA for WT and mutant, n = 8–11), excluding a dominant negative effect of the mutation (data not shown).

We did not observe a significant change in the voltage dependence or the kinetics of currents derived from 2513delG mutant channels when compared with the WT. The activation curve, as obtained from tail current amplitudes at −30 mV, revealed a half-maximal activation at −41 ± 1 mV for the WT and −37 ± 1 mV for the mutant, respectively (see Fig 2C). Current activation was well fit to a first-order exponential function yielding the time constant $\tau_{act.}$ (see Fig 2D). Deactivation was measured at various test potentials after a 2-second activating pulse to +20 mV (Fig 3A). Tail currents were also well fit to a first-order exponential throughout the whole voltage range, yielding the deactivation time constant $\tau_{deact.}$ (see Fig 3B). The reversal potential, as derived from a tail current analysis, was

Fig 2. Potassium current amplitude and activation parameters for wild-type (WT) and mutant KCNQ2 channels. (A) Representative raw current traces at −80 to +20 mV as recorded from Xenopus laevis *oocytes injected with either WT or mutant KCNQ2 cRNA and from an uninjected oocyte. The 4-second depolarizing test pulse was followed by a 0.5-second pulse to −30 mV to measure the tail current amplitude, before repolarization to −80 mV. Note the 10-fold different scaling for "G838X" and "WT" on the left versus "2513delG" and "uninjected" on the right side. (B) Current amplitudes determined at the end of a 4-second depolarizing test pulse to +10 mV for all three clones and a 1:1 mixture of each mutant with WT, when approximately the same total amount of cRNA was injected into each oocyte. Oocytes were injected and measured on the same day (see Materials and Methods). Statistically significant differences to the WT are indicated as follows:* $^{*}p < 0.05$, $^{**}p < 0.001$; *n = 12–16. (C) Activation curves for all three clones as obtained from tail current amplitudes at −30 mV. Lines represent fits to a standard Boltzmann function,* $I/I_{max}(V) = 1/(1 + exp[(V - V_{0.5})/k])$, *with* I/I_{max} *being the normalized potassium current amplitude,* $V_{0.5}$ *the voltage of half-maximal activation, and* k *a slope factor. Parameters were as follows:* $V_{0.5} = -41 \pm 1$ *mV,* -42 ± 1 *mV, and* -37 ± 1 *mV;* $k = 7.9 \pm 0.5$ *mV,* 5.9 ± 0.4 *mV, and* 7.0 ± 0.2 *mV for WT, G838X, and 2513delG, respectively, n = 13–22. (D) Time constant of activation,* $\tau_{act.}$, *as obtained by a first-order exponential fit to the rising phase of the potassium current at various test potentials, n = 9–15. The difference between G838X and WT was statistically significant (eg, at +10 mV:* $\tau_{act.} = 74 \pm 5$ *vs* 120 ± 16 *msec,* $p < 0.05$, *n = 10, 12).*



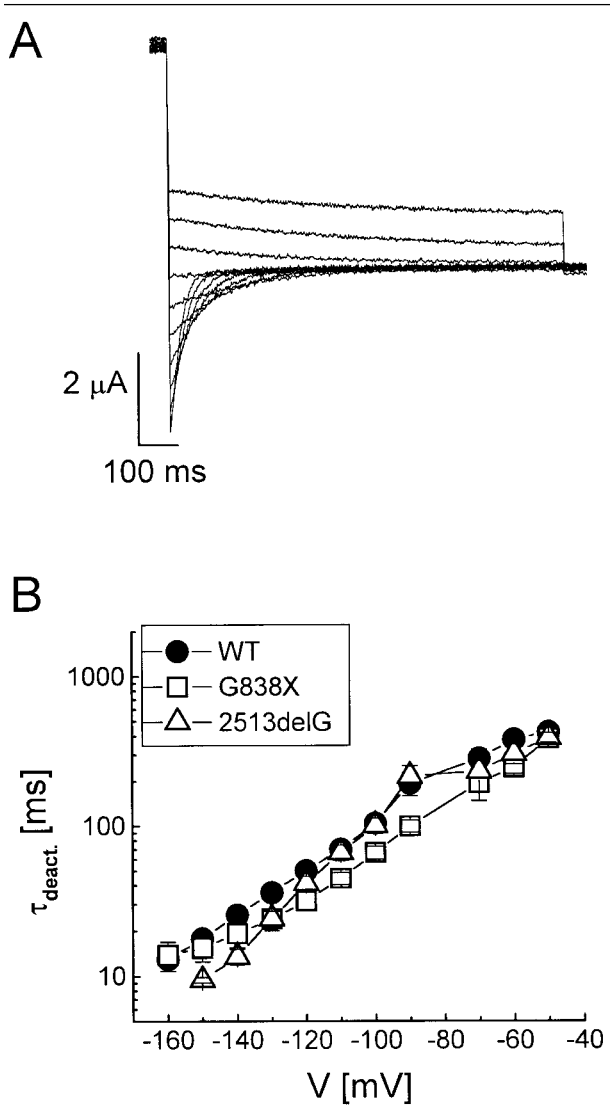


Fig 3. Deactivation kinetics for wild-type (WT) and mutant KCNQ2 channels. (A) Tail current traces recorded after a 2-second depolarization to +20 mV at test potentials between −50 and −160 mV from an oocyte injected with WT cRNA. (B) The tail current decay was fit to a first-order exponential function, yielding the deactivation time constant, $\tau_{deact.}$, n = 10–12. The difference between G838X and WT, seen almost throughout the whole voltage range, was not statistically significant (eg, at −100 mV, p = *0.0504).*

not significantly changed (−74 ± 2 mV for the WT vs −72 ± 2 mV for 2513delG), indicating that the channel pore was not affected by the mutation.

To evaluate if the additional 56 amino acids, or just the loss of the last seven regular residues, caused the reduction in current amplitude of the 2513delG mutant channel, we constructed a second mutation with a stop codon at position 838, that is, a protein truncated by the last seven C-terminal amino acids ("G838X"). It is noteworthy that this mutation yielded potassium currents with a twofold increase in amplitude compared with the WT (8.4 ± 1.1 μA, n = 12; see Fig 2A and B). In addition, G838X slightly accelerated activation and deactivation throughout the whole voltage range, albeit the latter not significantly (see Fig 1C and D and Fig 3B). The reversal potential was determined to −71 ± 2 mV, which is not significantly different from those of the two other clones. The data indicate that it is the pathological extension of the mutant protein, and not the loss of the last seven regular C-terminal amino acids, that produces the phenotype.

Discussion

A combination of clinical, genetic, and electrophysiological investigations has revealed that BFNC in the reported family is caused by a mutation within a poorly conserved region of KCNQ2 decreasing the repolarizing potassium current. From the clinical point of view, the family confirms previously reported data of BFNC concerning (1) the age of onset and disappearance, and the variable semiology of the seizures, (2) an individual recurring seizure type for 3 affected family members where detailed history was available, (3) the increased risk of recurring seizures later in life, (4) a normal or only unspecifically altered interictal EEG, (5) a normal physical and mental development, and (6) the high penetrance of the disease.[1–3] One affected individual had seizures only around the sixth month of life, but not in the neonatal period, which is typical for the clinically related syndrome of benign familial infantile convulsions (BFIC).[19,20] Thus, both syndromes show a certain overlap regarding their age of onset, which might indicate that BFIC is caused by a similar genetic defect, as has also been reported by Singh and associates.[7] So far, one locus for BFIC is described on chromosome 19q,[21] and a second one in the centromeric region of chromosome 16 for BFIC, going along with paroxysmal choreoathetosis,[22] but the genes are still unknown. In contrast, FCs are of different pathogenesis in our family, at least in the 3 individuals who experienced only FCs but did not carry the mutation, in accordance with Leppert and collaborators[4] and Biervert and co-workers.[8] However, because of the high incidence of FCs and the underlying heterogeneity, it cannot be excluded that the 2513delG mutation adds to the increased susceptibility for febrile seizures in family members affected by both BFNC and FCs (as for the index patient in our family, Individual IV-4). It has already been shown that FCs can be caused by an abnormal gating of ion channels, as reported for a rare dominantly inherited subset of FCs combined with generalized epilepsy.[13]

KCNQ2 encodes a delayed rectifier potassium channel that is activated after depolarization of the cell membrane and therefore contributes to the repolarizing phase of the action potential. A reduction of this cur-

rent impairs repolarization leading to a hyperexcitability of the cell membrane, which can explain the pathogenesis of seizures in BFNC, especially because KCNQ2 is expressed in most regions of the brain.[8,23] The same pathomechanism seems to be valid for an inherited cardiac arrhythmia, the long-QT syndrome, for which mutations have been found in KCNQ1, a heart muscle–specific potassium channel of the same family (see below). All mutations that have been functionally characterized thus far substantially reduce the resulting potassium current.[16,17] Additional evidence for the induction of seizures by a reduced potassium current comes from studies with potassium channel blockers in brain slices. Dendrotoxin, 4-aminopyridine, and tetraethylammonium, classic blockers of various types of potassium channels, all produce epileptic activity.[24–26]

KCNQ2 belongs to a new family of voltage-gated potassium channels, which are mutated in either the long-QT syndrome (KCNQ1) or BFNC (KCNQ2 and KCNQ3). These channels have a particularly long C-terminus containing several of the mutations that have been identified so far.[6–8,10,16,17,27] The functional role of this region is still unclear. Four of the mutations in the C-terminus, three in KCNQ1 and one in KCNQ2, have been functionally expressed thus far.[8,16,17] Only one point mutation in KCNQ1 predicting substitution of cysteine for a conserved arginine (R555C) showed a functional abnormality, namely, a large depolarizing shift of the activation curve, which might indicate interaction with the voltage sensor.[16] All other mutations induced frameshifts and premature stop codons resulting in truncated proteins that did not yield functional channels.

The particularity of the mutation reported here is that this mutation resides in the very distal, poorly conserved C-terminal domain. The mutation induces a rather unspecific change of the last seven and a tail of additional 56 amino acids. Nevertheless, the electrophysiological data presented here indicate that this mutation greatly reduces potassium current size. Moreover, truncation at the site of the mutation (ie, loss of the last seven amino acids) leads to increased potassium currents, indicating that also this part of the channel serves a precise, although yet unknown function in regulating channel expression.

We can only speculate on the mechanism of how currents are reduced in 2513delG. Because our results suggest normal pore properties, it is unlikely that this mutation affects single channel conductance. Also channel gating was unaffected, indicating that the mutated part does not interact with functionally important regions of the channel protein. Thus, there should be either an instability on the RNA level, impaired folding of the protein, or an insufficient transport/insertion to the cell membrane reducing the number of channels functionally available. Because potassium channel subunits coassemble to tetramers and we did not observe a significant dominant negative effect of the mutation, channel function should not significantly be suppressed by coassembly of mutant and WT subunits. Haploinsufficiency should therefore produce the phenotype. Nevertheless, a similar channel, KCNQ1, interacts with a β-subunit, minK, to produce the slow cardiac potassium current I_{Ks}.[28,29] The absence of a corresponding not yet identified β-subunit, which might interact with KCNQ2, may hide an obvious dominant negative effect.[8]

The question remains as to why the reduced KCNQ2 potassium current results in seizures preferentially during the neonatal period. One possibility could be that the brain is generally more likely to develop seizures in this premature state than later in life.[30] Another explanation might be the differential expression of potassium channels during maturation, which may attribute a dominant role to KCNQ channels in central neurons within the first days to weeks of life. Either potassium channels of the KCNQ family could be up-regulated during this period, or other voltage-gated potassium channels could still not have reached their full expression level. For some of the rat brain voltage-gated potassium channels, such a differential expression during maturation has already been described.[31] In electrophysiological experiments on hippocampal brain slices, changes of potassium currents have also been reported during ontogenesis.[32] It is noteworthy that even epileptic activity influenced the expression level of some potassium channels within hippocampal dentate granule cells.[33]

Note Added in Proof

Since this manuscript was submitted, two other papers concerning KCNQ2 and KCNQ3 channels appeared that require attention. The first, by Wang and colleagues,[34] shows that these channels constitute the "M-current," a potassium current that regulates neuronal excitability in many parts of the brain. These results fit perfectly with the pathophysiological concept, that a los of KCNQ-mediated potassium current results in epileptic seizures. The second paper, by Schroeder and coworkers,[35] presents similar results as in this article, showing a reduction in the resulting potassium current by other KCNQ mutations.

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