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Peripheral nerve hyperexcitability due to dominant-negative *KCNQ2* mutations



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ABSTRACT

Background: Peripheral nerve hyperexcitability (PNH) is characterized by muscle overactivity due to spontaneous discharges of lower motor neurons usually associated with antibodies against voltage-gated potassium channels. PNH may also occur in combination with episodic ataxia or epilepsy caused by mutations in $K_{v1.1}$ or $K_{v7.2}$ channels. Only one PNH-associated mutation has been described so far in $K_{v7.2}$ (R207W), in a family with both PNH and neonatal seizures.

Methods: PNH was characterized by video and electromyography. The *KCNQ2* gene was sequenced and $K_{v7.2}$ channels were functionally characterized using two-microelectrode voltage-clamping in *Xenopus* oocytes.

Results: In a patient with PNH without other neurologic symptoms, we identified a novel *KCNQ2* mutation predicting loss of a charged residue within the voltage sensor of $K_{v7.2}$ (R207Q). Functional analysis of both PNH-associated mutants revealed large depolarizing shifts of the conductance-voltage relationships and marked slowing of the activation time course compared to wild type (WT) channels, less pronounced for R207Q than R207W. Co-expression of both mutant with WT channels revealed a dominant negative effect reducing the relative current amplitudes after short depolarizations by >70%. The anticonvulsant retigabine, an activator of neuronal K_{v7} channels, reversed the depolarizing shift.

Conclusions: Mutations in *KCNQ2* can cause idiopathic PNH alone and should be considered in sporadic cases. Both $K_{v7.2}$ mutants produce PNH by changing voltage-dependent activation with a dominant negative effect on the WT channel. This distinguishes them from all hitherto examined $K_{v7.2}$ or $K_{v7.3}$ mutations which cause neonatal seizures by haploinsufficiency. Retigabine may be beneficial in treating PNH. *Neurology*® 2007;69:2045-2053

GLOSSARY

BFNC = benign familial neonatal convulsions; **EA-1** = episodic ataxia with myokymia; **EMG** = electromyography; **PNH** = peripheral nerve hyperexcitability; **VGKC** = voltage-gated potassium channels; **WT** = wild type.

Peripheral nerve hyperexcitability (PNH) comprises a heterogeneous group of diseases.¹⁻⁸ According to their etiology, autoimmune-mediated vs non-autoimmune-mediated forms, including genetic disorders, motor neuron degeneration, intoxication, and axonal trauma (e.g., radiation), can be distinguished. Clinically they are characterized by a spontaneous and continuous muscle overactivity, which has been described as myokymia (continuous undulating movements of distal skeletal muscle), fasciculations, cramps, stiffness, pseudomyotonia, normocalcemic or pseudotetany, or a combination of these symptoms.^{2,3} Additionally, some patients have sensory (numbness, paraesthesias) or autonomic symptoms (constipation, urine incontinence, hyperhidrosis). Electrophysiologically, two groups with or without myokymic discharges in electromyography (EMG) can be distinguished. These discharges are character-

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ized by doublet, triplet, or multiplet motor unit or partial motor unit potentials, which predict more severe and more diverse motor symptoms. However, the electrophysiologic phenotype can change with time.³

PNH is commonly caused by a loss of function of voltage-gated potassium channels (VGKC) resulting in a decreased potassium outward current.⁹ VGKCs regulate neuronal firing by contributing to the repolarizing phase of the action potential and by setting the membrane potential in the subthreshold range.¹⁰ In autoimmune mediated PNH, loss of VGKCs is caused in fewer than 50% by antibodies against these channels. Furthermore, ion channel mutations may cause PNH in conjunction with a complex phenotype such as episodic ataxia with myokymia (EA-1) for which mutations in the gene *KCNA1*, encoding the human homolog of the *Shaker* potassium channel $K_V1.1$, have been identified.¹¹⁻¹⁴ For PNH combined with neonatal epilepsy, a single mutation in the *KCNQ2* gene encoding the potassium channel $K_V7.2$ has been reported.¹⁵

Neuronal K_V7 channels generate the M-current, a non-inactivating potassium current which is particularly important to regulate excitability in many neurons by influencing the subthreshold membrane potential.¹⁶ Many mutations have been described in $K_V7.2$ channels, and a few ones in $K_V7.3$, to cause benign familial neonatal convulsions (BFNC), an autosomal dominantly inherited epilepsy syndrome characterized by seizures in the first days and weeks of life.¹⁷ Functional analysis of these BFNC-causing mutations revealed a more or less complete loss of function without a prominent dominant negative effect on wild type (WT) channels. Therefore, a haploinsufficiency is commonly believed to induce seizures in BFNC.¹⁸⁻²⁴ Although these channels are not only detected in the CNS but also highly expressed in peripheral nerves,^{15,25} only one of the many mutations is associated with clinically manifest PNH in addition to BFNC. This mutation predicts the loss of a positive charge within the $K_V7.2$ channel's voltage sensor (R207W) and has a unique functional consequence: it

leads to a large positive shift of voltage-dependent activation resulting in a pronounced dominant negative effect on the WT channel for the first 200 msec after onset of the depolarization.¹⁵

Here we describe a second mutation at the same position in the voltage sensor region of $K_V7.2$, being associated with sporadic PNH without evidence for neonatal seizures in a single patient of Egyptian origin, and without evidence for PNH or BFNC in his family. We present a video of typical myokymia in the distal upper limbs, typical electromyographic recordings, and a detailed functional analysis of both $K_V7.2$ mutations associated with PNH. Our results emphasize the important role of $K_V7.2$ channels at subthreshold membrane potentials and suggest a distinct sensitivity of peripheral and central neurons to a reduction of the M-current in different stages of development. Furthermore, we provide evidence for a potentially therapeutic effect of the new anticonvulsant compound retigabine, a specific K_V7 channel activator, on PNH.

METHODS Subjects. All patients and their unaffected relatives (or their legal representatives) gave written informed consent to participate in the study. All studies conformed to the standards set by the Declaration of Helsinki, and all procedures were approved by the Ethical Committee of the University of Ulm, Germany. The index patient was personally interviewed and examined by experienced neurologists (F.L.H., W.P.).

Mutation analysis. Genomic DNA was extracted by standard methods. The coding regions and exon-intron boundaries of *KCNQ2*²² and of the single exon encoding *KCNA1* were amplified by PCR using previously published PCR primers. Gel purified products were automatically sequenced and patient sequences were compared to published sequences for *KCNQ2* (GenBank, NM_172107) and *KCNA1* (GenBank, NM_000217).

Mutagenesis and RNA preparation. Site-directed mutagenesis using PCR techniques was performed to introduce both mutations in the *KCNQ* cDNA cloned in the pTLN vector (primers are available upon request). Both mutations were verified by automated DNA sequencing. Plasmids were linearized by digestion with the restriction enzyme MluI. Linearized plasmids were transcribed in vitro using the SP6 mMessage mMachine kit (Ambion Inc., Austin, TX) resulting in capped cRNA. Purity was checked by gel electrophoresis. Concentration was verified by spectrophotometry.

Oocyte preparation and injection. All procedures met the NIH guidelines for the care and use of laboratory animals and were approved by the Regierungspräsidium

Tuebingen, Germany. Tricaine (0.1%; Sigma, Deisenhofen, Germany) was used to anesthetize female *Xenopus laevis* frogs. Oocytes were surgically removed and treated for 2 hours by collagenase (2 mg/mL of type CLS III collagenase, Biochrom KG, Berlin, German) in OR2-solution (mM: 82.5 NaCl, 2.5 KCl, 1 MgCl₂ and 5 Hepes, pH 7.6) in order to remove follicular structures. Defolliculated oocytes were stored at 16 °C in frog Ringer solution (in mM: 115 NaCl, 2.5 KCl, 1.8 CaCl₂ and 10 Hepes, pH 7.4) supplemented with 50 µg/mL gentamicin (Biochrom KG). Diluted cRNA (10 to 20 ng) was injected into each oocyte within 24 hours after preparation. Electrophysiologic recordings were performed in frog Ringer solution at room temperature (21 to 23 °C) 3 days after injection.

Electrophysiology and data evaluation. Potassium currents were recorded using standard two-microelectrode voltage clamping, a Turbo TEC01C amplifier (npi electronic GmbH, Tamm, Germany), and pClamp data acquisition (Axon instruments, Foster City, CA), as described previously.²⁶ Frog Ringer (see above) was used as the bathing solution for all recordings. Recording electrodes were filled with 3 M KCl and had a resistance of 0.3 to 1 MΩ. Currents were low-pass filtered at 0.3 kHz and sampled at 1 kHz. Oocytes were clamped to a holding potential of -80 mV followed by depolarizing pulses in 10 mV steps up to +60 mV. Due to the slow time course of activation in the mutants, pulses with a duration of up to 5 s were used. Tail currents were recorded at -30 mV and their amplitudes analyzed to obtain conductance-voltage plots. To evaluate the current reduction after shorter depolarizations, current amplitudes were analyzed after 200 msec and normalized to those at the end of a 5-s (mutant) or 2-s (WT) depolarization to +60 mV (maximal activation).

Experiments with retigabine (RGB) were performed as described previously.²⁷ Oocytes were clamped to a holding potential of -100 mV and depolarized in 20-mV steps up to +20 mV. Solutions with different concentrations of retigabine (0 µM, 1 µM, 10 µM, 100 µM) were used to obtain dose-response curves. RGB caused a hyperpolarizing shift of the steady-state activation curve. $V_{0.5} - V_{0.5} (+RGB)$ was plotted against the logarithm of the RGB concentration. A Hill function was fit to the data points according to the following equation:

$$V_{0.5} - V_{0.5} (+RGB) = A_2 + [(A_1 - A_2)/(1 + (x/x_0)^{n_H})]$$

where $V_{0.5} - V_{0.5} (+RGB)$ represents the difference in $V_{0.5}$ of the activation curve (see below) in presence and absence of RGB, A_1 is the minimal and A_2 the maximal hyperpolarizing shift, x the concentration of RGB, x_0 the EC₅₀ (concentration of RGB resulting in the half-maximal hyperpolarizing shift of the activation curve), and n_H the slope factor (Hill coefficient) of the curve.

Data were analyzed using pClamp, Microsoft Excel (Microsoft, Redmond, WA), and Origin (Microcal Software, Northampton, MA) software. A Boltzmann equation was fit to conductance-voltage relationships (activation curves):

$$I/I_{\max} (V) = 1/(1 + \exp[(V - v_{0.5})/k])$$

with I/I_{\max} being the normalized tail current amplitude, $V_{0.5}$ the voltage of half-maximal activation, and k a slope factor. Time constants of activation and deactivation were obtained by fitting a first order exponential function to the rising part of the current traces or to the tail current decay.

For statistical evaluation a two-tailed, unpaired Student *t*-test was applied ($p < 0.05$ was considered to be significant). All data are shown as means ± SEM.

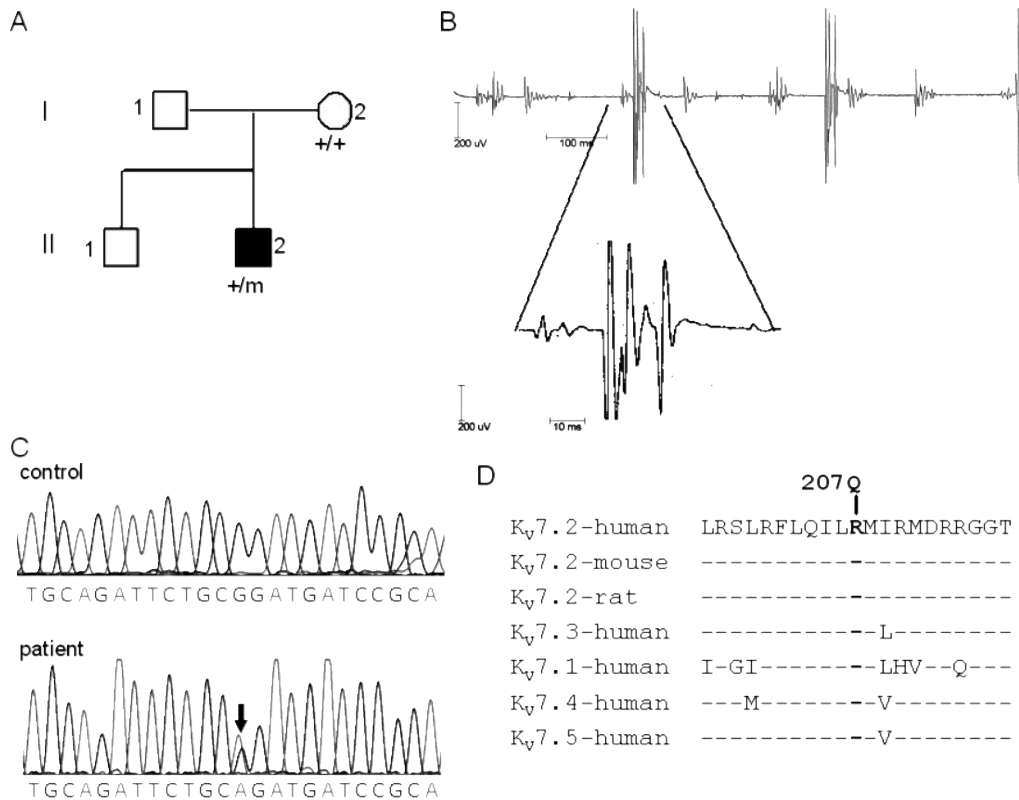
RESULTS Clinical data. The index patient of the Egyptian family is the only individual affected by PNH (figure 1A). He has no history of neonatal seizures. He was 25 years old at the time that studies were performed. He presented with a permanent muscle overactivity which was best visible in the distal upper extremities as small amplitude movements of his fingers. A video of these spontaneous movements in the patient's right hand can be seen on the *Neurology*[®] Web site. These involuntary movements are not disabling for him. He denied problems with writing, typing, or other motor skills using the hands. The patient could not tell if the involuntary movements also occur during sleep. He was not willing to come overnight to the hospital for monitoring or other further investigations. In addition to the permanent overactivity, the patient reported exercise-induced cramps primarily of both hands since the age of 6 years. Extensive physical exertion and anxiety can lead to periods with severe generalized muscle stiffness lasting up to 30 minutes, which happened four times in his life. These events were threatening for him and he therefore avoids heavy exercising. Both the index patient and his mother denied any symptoms which could be attributed to neonatal or other epileptic seizures during his life. His neurologic examination was otherwise unremarkable, including absence of muscle hypertrophy and sensory symptoms. Treatment with carbamazepine was unsuccessful and was discontinued due to a rash, but lamotrigine in a dosage of 200 mg per day was of some benefit.

Electromyography of thenar muscles revealed spontaneous discharges mainly in form of irregular multiplets (figure 1B), consistent with myokymic discharges as a typical phenotype of PNH.³ Motor and sensory nerve conduction studies revealed normal results.

None of the other family members, who all live in Egypt, was reported to have experienced symptoms of PNH or BFNC.

Molecular genetic analysis. Blood samples were obtained from the index patient and from his mother during a short visit in Germany. All other family members refused a genetic analysis. While sequencing of the *KCNA1* gene did not reveal any changes, the *KCNQ2* gene revealed a heterozygous point mutation predicting the loss of a charged residue in the voltage sensor (segment S4) of the K_v7.2 channel (substitution of glutamine for the third of six highly conserved arginines,

Figure 1 Clinical and genetic findings



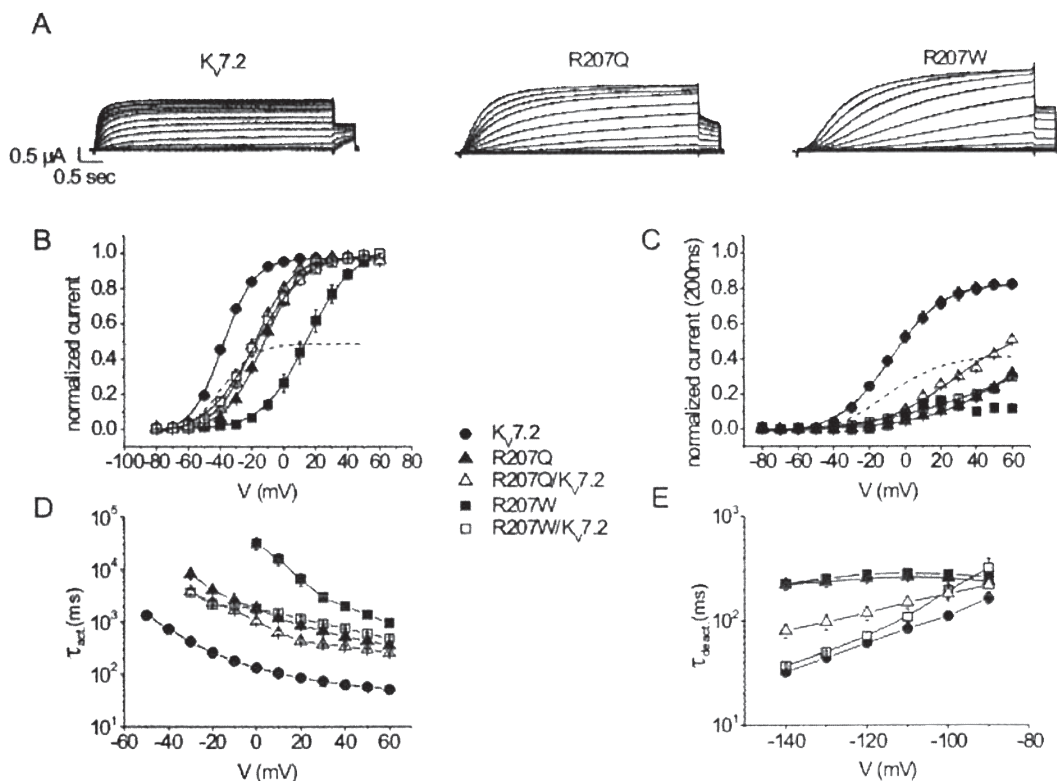
(A) Pedigree of the index patient indicating that there was no hint of PNH in other family members and that the mutation detected in the patient but not in his mother might have occurred de novo. (B) Typical EMG recordings from the index patient, revealing spontaneous activity of the M. abductor pollicis brevis. (C) Sequence of genomic DNA showing the heterozygous missense mutation at codon 207. (D) R207 is located within the S4 transmembrane domain of K_V7.2 and is conserved in the orthologous K_V7.2 protein of human, mouse, and rat as well as in human K_V7.3, K_V7.1, K_V7.4, and K_V7.5. Analogous positions to R207 are marked in bold. GenBank accession numbers are (from top): AY889405, AF490773, AF087453, AF071491, AY114213, AF105216, AF249278.

R207Q (figure 1, C and D). His mother did not carry the mutation. Further mutations in *KCNQ2* were not detected by sequencing all exons including adjacent splice sites. The mutation was excluded in 98 healthy controls.

Electrophysiology. To characterize the functional consequences of the two only *KCNQ2* mutations identified up to now to be associated with PNH, the one described previously by Dedek et al.,¹⁵ R207W, and the new one found in this study, R207Q, both were heterologously expressed as homomers and in various combinations with K_V7.2 and K_V7.3 in *Xenopus laevis* oocytes. Potassium currents were recorded using standard two-microelectrode voltage clamping. Oocytes were depolarized from a holding potential of -80 mV in 10 mV steps up to $+60$ mV followed by a pulse to -30 mV to record tail currents and construct conductance-voltage curves. Raw current traces are shown in figure 2A and figure 3A revealing a prominent slowing of the activation

time course for both mutations compared to the WT. Maximal current amplitudes of the homomeric and heteromeric mutant channels were not decreased in comparison to WT channels (examples are shown in figure 2A and figure 3A). Conductance-voltage curves were strongly shifted, by $+25$ mV for R207Q and $+53$ mV for R207W channels, as compared to K_V7.2 WT channels (figure 2B). Co-expression studies of mutant with WT K_V7.2 channels in a 1:1 ratio demonstrated a reduction of the normalized steady-state conductance at subthreshold potentials between -50 and -30 mV which was slightly stronger than 50%, as would be expected for a haploinsufficiency (figure 2B dashed line). Thus even in the steady state, we obtained evidence for a mild dominant negative effect of both mutants upon co-expression with WT K_V7.2 channels. However, spontaneous discharges of peripheral motor neurons will hardly involve 5 s lasting depolarizations (compare figure 1B) and

Figure 2 Functional characterization of WT ($K_v7.2$) and mutant (R207Q, R207W) channels



(A) Representative raw current traces for homomeric $K_v7.2$, R207Q, and R207W channels. Currents were elicited from a holding potential of -80 mV by depolarizations ranging from -80 to $+60$ mV in 10 mV steps, and tail currents were recorded at -30 mV before repolarization. (B) Conductance-voltage curves were constructed by plotting the normalized tail current amplitude recorded at -30 mV against the membrane potential. Lines represent standard Boltzmann functions fit to the data points as described in Methods. Parameters were as follows: $K_v7.2$: $V_{0.5} = -38.3 \pm 0.9$ mV, $k = -9.3 \pm 0.3$ mV; R207Q: $V_{0.5} = -12.7 \pm 1.2$ mV, $k = -11.3 \pm 0.3$ mV; R207Q/ $K_v7.2$: $V_{0.5} = -18.8 \pm 1.2$ mV, $k = -11.3 \pm 0.5$ mV; R207W: $V_{0.5} = 14.9 \pm 3.5$ mV, $k = -11.4 \pm 0.4$ mV; R207W/ $K_v7.2$: $V_{0.5} = -17.6 \pm 3.0$ mV, $k = -14.7 \pm 1.0$ mV, exhibiting a strong depolarizing shift of the activation curves of R207Q ($n = 21$; $p < 1$ E-15), R207Q/ $K_v7.2$ ($n = 7$; $p < 1$ E-9), R207W ($n = 12$; $p < 1$ E-13), and R207W/ $K_v7.2$ ($n = 5$; $p < 1$ E-7) in comparison to $K_v7.2$ ($n = 14$). Comparison to the assumed 50% current reduction in case of a haploinsufficiency of $K_v7.2$ (dashed line) predicted a dominant negative effect for both R207Q and R207W when co-expressed with $K_v7.2$ at subthreshold potentials (R207Q/ $K_v7.2$: -50 mV: $p < 0.001$; -40 mV: $p < 1$ E-5; R207W/ $K_v7.2$: -50 : $p < 0.05$; -40 : $p < 0.01$). Data are shown as means \pm SEM. (C) To evaluate the current reduction after short-term depolarizations, amplitudes of currents mediated by mutant channels were determined after 200 msec and normalized to the current amplitude at $+60$ mV after a 5 s depolarization and compared to the respective amplitudes for the WT normalized to the maximum amplitude reached after 2 s at $+60$ mV. Relative current amplitudes of the co-expressions R207Q/ $K_v7.2$ and R207W/ $K_v7.2$ were reduced in comparison to $K_v7.2$ for a broad range of potentials and were as follows at e.g., -40 mV: $K_v7.2$: 0.039 ± 0.007 ; R207Q/ $K_v7.2$: 0.000 ± 0.002 ($p < 0.01$); R207W/ $K_v7.2$: 0.010 ± 0.006 ($p < 0.05$). Comparison to the assumed 50% reduction of current in case of a haploinsufficiency of the $K_v7.2$ WT ($n = 9$) (dashed line) predicted a strong dominant negative effect for both R207Q and R207W when co-expressed with $K_v7.2$ ($n = 6, 4$) with a $> 70\%$ reduction of the relative current amplitude for potentials between -40 and $+10$ mV. Parameters are given as means \pm SEM. (D) Time constants of activation (τ_{act}) for $K_v7.2$ ($n = 10$), R207Q ($n = 10$), R207W ($n = 10$) and the co-expressions R207Q/ $K_v7.2$ ($n = 7$), R207W/ $K_v7.2$ ($n = 5$) were obtained by fitting a first order exponential function to the rising part of each current trace. Means for $\tau_{act} \pm$ SEM were plotted against voltage, revealing a pronounced slowing of activation kinetics for both mutants. Activation time constants were different for all plotted potentials ($p < 1$ E-7). (E) Time constants of deactivation (τ_{deact}) of $K_v7.2$ ($n = 8$), R207Q ($n = 8$), R207W ($n = 5$), and the co-expressions R207Q/ $K_v7.2$ ($n = 8$), R207W/ $K_v7.2$ ($n = 4-5$) were evaluated by fitting a first order exponential function to the tail current decay at different potentials after a 5-second lasting depolarizing pulse to $+50$. Data for τ_{deact} were plotted against voltage and are shown as means \pm SEM.

the strong slowing of the activation time course predicted a much larger reduction of current amplitudes after short-lasting depolarizations. To evaluate this effect, amplitudes of currents conducted by mutant channels as measured 200 msec after onset of the depolarization were normalized

to the maximum amplitude reached after 5 s at $+60$ mV and compared to the respective amplitudes of the WT normalized to the maximum amplitude reached after 2 s at $+60$ mV. Using this method, we observed a very strong dominant negative effect of both mutations on WT $K_v7.2$ chan-

nels for a physiologically relevant time range with a >70% reduction of current amplitudes at potentials between -40 and +10 mV when compared to $K_{V7.2}$ channels alone (figure 2C). The marked slowing of the activation time course for R207W > R207Q and the co-expressions with $K_{V7.2}$ WT channels are shown in figure 2D. Deactivation kinetics showed a loss of voltage dependence for homomeric mutant channels but were only slightly slowed for heteromeric R207Q/ $K_{V7.2}$ and R207W/ $K_{V7.2}$ channels (figure 2E).

Heteromeric $K_{V7.2}/K_{V7.3}$ channels have much larger amplitudes compared to the individual channels alone and could represent the most abundant morphologic correlate of the M-current in the mammalian brain.^{16,28,29} Taking into account a potential colocalization of $K_{V7.2}$ and $K_{V7.3}$ subunits also in peripheral motor neurons, we evaluated the functional consequences of the R207Q and R207W mutants when co-expressed with $K_{V7.3}$ channels. We compared currents in oocytes injected with the same total amount of cRNA 1) for a 1:1 co-expression of $K_{V7.2}$ and $K_{V7.3}$ WT channels, 2) for R207Q or R207W with $K_{V7.3}$ in a 1:1 ratio, and 3) for each of the mutants with $K_{V7.2}$ and $K_{V7.3}$ in a 1:1:2 ratio (to mimic the potential situation in an affected individual carrying one mutant *KCNQ2*, one WT *KCNQ2*, and two WT *KCNQ3* alleles). The effects observed under these conditions were similar to those with a $K_{V7.2}$ co-expression but less pronounced for R207Q. Conductance-voltage curves in the steady-state for co-expressions of each of the mutants with $K_{V7.3}$ showed clear depolarizing shifts for R207W and R207Q compared to $K_{V7.2}/K_{V7.3}$ WT channels; the effect was much more pronounced for R207W (figure 3B). This shift was still statistically significant for the co-expression of R207W/ $K_{V7.2}/K_{V7.3}$ channels, but there was no significant difference for R207Q/ $K_{V7.2}/K_{V7.3}$ compared to $K_{V7.2}/K_{V7.3}$ alone (figure 3B). A similar scenario was observed for the current-voltage relationship when current amplitudes were analyzed 200 msec after onset of the depolarization (figure 3C). Except for R207Q/ $K_{V7.2}/K_{V7.3}$, activation time constants (τ_{act}) were increased compared to $K_{V7.2}/K_{V7.3}$ channels (figure 3D). Deactivation time constants were slowed for all co-expressions (figure 3E).

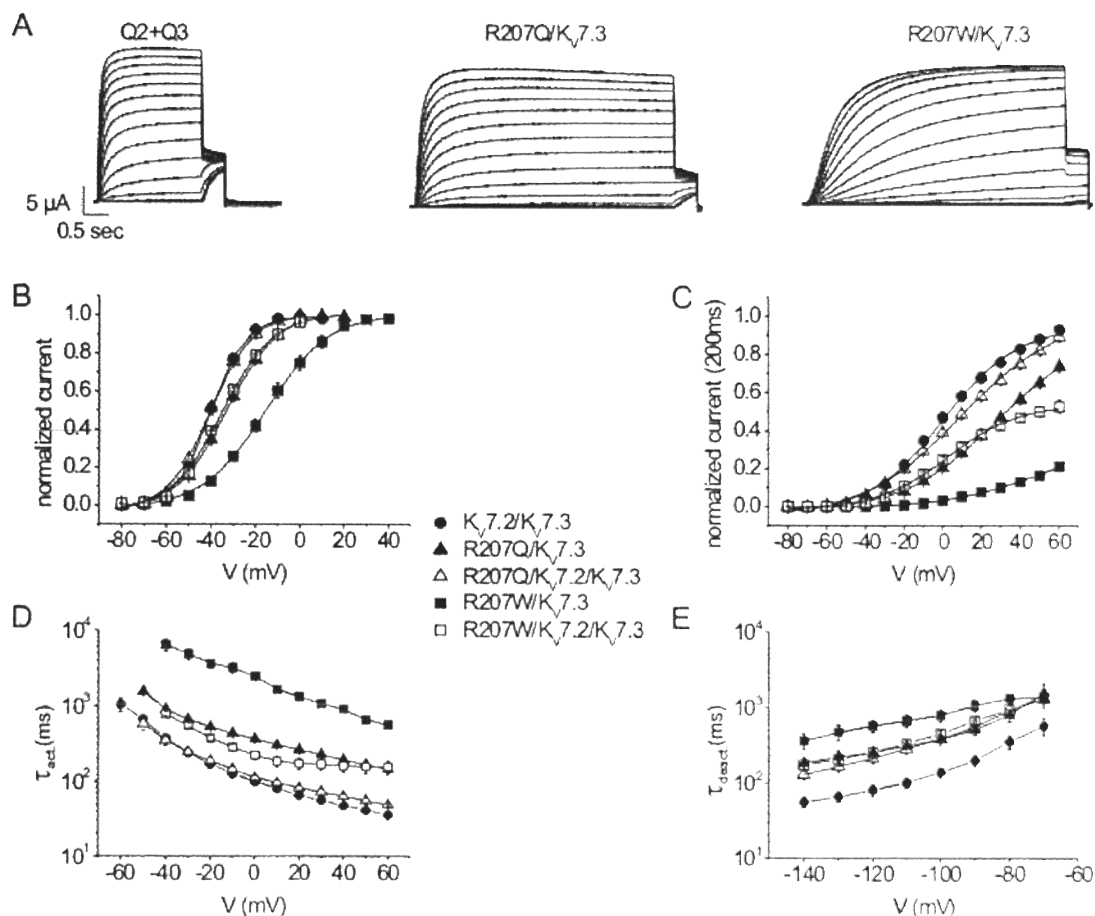
Recordings before and after application of different concentrations of retigabine, a new anticonvulsant drug activating neuronal K_{V7} channels, revealed a concentration-dependent hyperpolarizing shift of the activation curves (figure 4B): 25 mV for $K_{V7.2}$ WT and 27 mV for R207Q after washing

in of 100 μ M retigabine (figure 4A). This effect resulted in an increase of the relative current amplitude over a broad range of membrane potentials (figure 4A inset). The retigabine sensitivity was not altered for the mutation (figure 4B), indicating that the decrease in channel activity could be largely rescued by this drug.

DISCUSSION *KCNQ2* and *KCNQ3* mutations usually cause BFNC without PNH, although $K_{V7.2}$ and $K_{V7.3}$ channels are also expressed within the peripheral nervous system.^{15,25} All functional studies of these mutations revealed a haploinsufficiency of the *KCNQ2* or the *KCNQ3* gene as a common pathogenetic mechanism for BFNC.²²⁻²⁴ A 50% reduction of potassium currents carried by $K_{V7.2}$ channels or an about 25% reduction of those carried by heteromeric $K_{V7.2}/K_{V7.3}$ channels seems thus not to be sufficient to generate PNH. In contrast, functional analysis of the R207W mutation causing BFNC with PNH and of the R207Q mutation causing PNH alone establishes a different mechanism: after short depolarizations, both R207 mutants exert a strong dominant negative effect on co-expressed WT $K_{V7.2}$ channels brought on by a drastic depolarizing shift of the activation curve and a prominent slowing of the activation time course. Such a suppression of $K_{V7.2}$ -mediated currents may well explain a hyperexcitability of peripheral motor neurons.

PNH is generated in the distal motor neurons, since lidocaine block of the proximal nerves does not suppress this activity, as was also shown in patients carrying the R207W mutation.^{3,15} This is suggested as well by the typical variable morphology of the electromyographic discharges observed in our patient (figure 1B). Thus, spontaneous depolarizations of presumably short duration at nodes of Ranvier and maybe near the nerve terminals, where $K_{V7.2}$ channels are probably localized,^{25,30,31} may lead to spontaneous action potentials when activation of $K_{V7.2}$ channels is largely suppressed by dominant mutations. Interestingly, $K_{V7.2}$ and $K_{V7.3}$ may show a different subcellular distribution in peripheral motor neurons: $K_{V7.2}$ immunoreactivity was found in nodes of Ranvier, whereas the one for $K_{V7.3}$ was found clearly outside of nodes in rat sciatic nerves.²⁵ Thus, the 1:1:2 co-expression experiment, which did not reveal a difference compared to WT for the R207Q mutant in contrast to R207W, might not be relevant for the hyperexcitability of peripheral motor neurons. However, this result could serve as one explanation for the nonpenetration of neonatal seizures in the patient carry-

Figure 3 Heterologous expression of heteromeric WT $K_v7.2/K_v7.3$ channels, mutant R207Q/ $K_v7.3$, R207W/ $K_v7.3$, and R207Q/ $K_v7.2/K_v7.3$, R207W/ $K_v7.2/K_v7.3$ channels

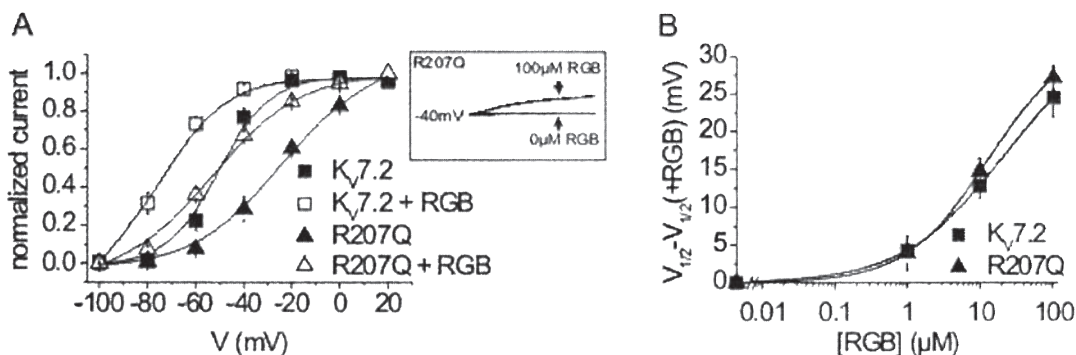


(A) Representative raw current traces for co-expressions as indicated. Currents were recorded as in figure 2A. (B) Conductance-voltage curves for co-expressions as indicated. cRNA was injected in either a 1:1 or a 1:1:2 ratio (see text). Lines represent standard Boltzmann functions fit to the data points as described in Methods. Parameters were as follows: $K_v7.2/K_v7.3$: $V_{0.5} = -40.0 \pm 1.0$ mV, $k = -7.7 \pm 0.2$ mV; R207Q/ $K_v7.3$: $V_{0.5} = -33.2 \pm 1.3$ mV, $k = -10.7 \pm 0.3$ mV; R207W/ $K_v7.3$: $V_{0.5} = -14.9 \pm 2.2$ mV, $k = -14.0 \pm 1.4$ mV; R207Q/ $K_v7.2/K_v7.3$: $V_{0.5} = -40.6 \pm 0.8$ mV, $k = -8.8 \pm 0.3$ mV; R207W/ $K_v7.2/K_v7.3$: $V_{0.5} = -35.2 \pm 0.3$ mV, $k = -10.7 \pm 0.4$ mV. The depolarizing shifts of the activation curves of all co-expressions were statistically significant in comparison to $K_v7.2/K_v7.3$ channels (R207Q/ $K_v7.3$ and R207W/ $K_v7.2/K_v7.3$ $p < 0.01$; R207W/ $K_v7.3$ $p < 1 \text{ E-}5$), except for R207Q/ $K_v7.2/K_v7.3$. Data are given as means \pm SEM, $n = 5-7$. (C) Current-voltage relationships for short-term depolarizations, as shown in figure 2C. Comparison to $K_v7.2/K_v7.3$ revealed a current reduction for R207Q/ $K_v7.3$, R207W/ $K_v7.3$, and R207W/ $K_v7.2/K_v7.3$ for a broad range of potentials between -50 and $+60$ mV and for R207Q/ $K_v7.2/K_v7.3$ for a smaller range of potentials between 0 and $+40$ mV. Relative current amplitudes at -50 mV were as follows: $K_v7.2/K_v7.3$: 0.014 ± 0.003 ; R207Q/ $K_v7.3$: 0.005 ± 0.001 ($p < 0.05$); R207W/ $K_v7.3$: 0.001 ± 0.001 ($p < 0.01$); R207Q/ $K_v7.2/K_v7.3$: 0.018 ± 0.001 (not significant); R207W/ $K_v7.2/K_v7.3$: 0.004 ± 0.001 ($p < 0.05$). Parameters are given as means \pm SEM, $n = 5-6$. (D) Time constants of activation (τ_{act}) for $K_v7.2/K_v7.3$ ($n = 6$), R207Q/ $K_v7.3$ ($n = 11$), R207W/ $K_v7.3$ ($n = 5$), R207Q/ $K_v7.2/K_v7.3$ ($n = 6$), and R207W/ $K_v7.2/K_v7.3$ ($n = 5$) channels were obtained as described in the legend to figure 2D and plotted as means \pm SEM. Activation kinetics of all co-expressions were slowed for all shown potentials in comparison to $K_v7.2/K_v7.3$ channels, except for R207Q/ $K_v7.2/K_v7.3$ (at -40 mV for example: R207Q/ $K_v7.3$ and R207W/ $K_v7.3$: $p < 0.001$; R207W/ $K_v7.2/K_v7.3$: $p < 1 \text{ E-}4$). (E) Time constants of deactivation (τ_{deact}) of $K_v7.2/K_v7.3$, R207Q/ $K_v7.3$, R207W/ $K_v7.3$, and R207Q/ $K_v7.2/K_v7.3$, R207W/ $K_v7.2/K_v7.3$ channels were evaluated as described in the legend to figure 2E and are shown as means \pm SEM, $n = 5-7$.

ing the R207Q mutation, since a close co-localization was detected for $K_v7.2$ and $K_v7.3$ in axon initial segments of pyramidal neurons of the hippocampus and cortex.²⁵ Another reason for the lack of neonatal seizures of the patient presented here might be simply a different genetic background compensating the hyperexcitability of the CNS in the neonatal period.

Our findings also correlate with the molecular level, on which it is conceivable that the bulky tryptophan residue causes a more severe disruption of the alpha-helical structure of the S4 segment than the more conservative change to a glutamine which at least has a similar size as arginine and preserves the alpha-helix. Since voltage-gated K^+ channels form tetramers,^{32,33} the observed dominant effect of

Figure 4 Effect of the K_{V7} opener retigabine on the R207Q mutation



(A) Conductance-voltage curves of homomeric $K_{V7.2}$ and R207Q channels were constructed before and after application of 100 μ M retigabine (RGB) as described in the legend to figure 2B. Retigabine led to a hyperpolarizing shift of the voltage of half-maximal activation, $V_{0.5}$, for both WT and mutant channels. Parameters were as follows: $K_{V7.2}$: $V_{0.5} = -50.3 \pm 2.1$ mV; $V_{0.5}(+RGB) = -74.9 \pm 2.7$ mV; R207Q: $V_{0.5} = -26.1 \pm 1.9$ mV; $V_{0.5}(+RGB) = -53.3 \pm 1.9$ mV ($p < 1 \times 10^{-5}$ for both conditions). The inset depicts a current trace of R207Q elicited at -40 mV before and after application of 100 μ M retigabine, clearly demonstrating an increment of the current by retigabine. (B) Dose-response curves for the activation of $K_{V7.2}$ WT and R207Q channels by retigabine. The relative hyperpolarizing shift of the voltage of half-maximal activation, $V_{0.5}$, was plotted against the logarithm of different concentrations of RGB. Lines represent a fit of the Hill function to the data points as described in Methods. The Hill coefficient, nH , was 0.6 ($K_{V7.2}$) and 0.8 (R207Q), respectively. Data are shown as means \pm SEM, $n = 6-8$.

the mutant subunits could be explained by the cooperativity of the four voltage sensors during channel opening.³⁴⁻³⁶

PNH is commonly treated symptomatically with anticonvulsant drugs. Retigabine is an anticonvulsant specifically enhancing K_{V7} channel activity, which is currently under investigation in phase III clinical trials for the treatment of focal epilepsy. Retigabine induces the opposite of the two myokymia-causing mutations, that is a hyperpolarizing shift of the activation curve of K_{V7} channels resulting in a stabilization of the membrane potential.³⁷⁻³⁹ Our results clearly showed that the pronounced depolarizing shift of the activation curve caused by the R207Q mutation could be reversed by retigabine resulting in an increased current amplitude at physiologically relevant subthreshold voltages. This is in agreement with the molecular mechanism of retigabine, since the negative shift of the activation curve was shown to be caused most probably by binding to the activation gate of K_{V7} channels and not the voltage sensor.^{27,40} The drug should not only have a positive influence on $KCNQ2$ -related PNH, but also be of benefit in other forms of the disease, since the enhancement of K_{V7} channels should generally reduce the firing of peripheral motor neurons. We therefore propose to consider retigabine as a new therapeutic option in PNH.

The electromyographic and clinical pattern of PNH observed in our patient was similar to those described in patients carrying the R207W mutation.¹⁵ All patients presented with permanent myokymia, in particular finger twitching as

shown in the supplementary video, and with typical myokymic discharges on EMG (figure 1B). An early onset and a lack of progression, which are not typical for autoimmune-mediated PNH, may reflect hallmarks of a $KCNQ2$ -related disorder, while the motor signs themselves represent a common phenotype observed also in other forms of PNH with distinct molecular etiologies.² Our results show that $KCNQ2$ mutations can cause idiopathic PNH alone, so that this gene should be considered for molecular diagnosis, also in sporadic cases without a family history. Finally, retigabine might become a new treatment option in PNH, not only for $KCNQ2$ -related cases.

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REFERENCES

1. Auger RG, Daube JR, Gomez MR, Lambert EH. Hereditary form of sustained muscle activity of peripheral nerve origin causing generalized myokymia and muscle stiffness. *Ann Neurol* 1984;15:13-21.
2. Hart IK, Newsom-Davis J. Generalized peripheral nerve hyperexcitability (neuromyotonia). In: Engel A, Franzini-Armstrong C, eds. *Myology: basic and clinical*, 3rd ed. New York: McGraw-Hill Medical Pub. Division; 2004:1301-1309.
3. Hart IK, Maddison P, Newsom-Davis J, Vincent A, Mills KR. Phenotypic variants of autoimmune peripheral nerve hyperexcitability. *Brain* 2002;125:1887-1895.

4. Isaacs H. A syndrome of continuous muscle-fibre activity. *J Neurol Neurosurg Psychiatry* 1961;24:319–325.
5. Liguori R, Vincent A, Clover L, et al. Morvan's syndrome: peripheral and central nervous system and cardiac involvement with antibodies to voltage-gated potassium channels. *Brain* 2001;124:2417–2426.
6. Mertens HG, Zschocke S. [Neuromyotonia]. *Klin Wochenschr* 1965;43:917–925.
7. Newsom-Davis J, Mills KR. Immunological associations of acquired neuromyotonia (Isaacs' syndrome). Report of five cases and literature review. *Brain* 1993;116(Pt 2):453–469.
8. Tahmouh AJ, Alonso RJ, Tahmouh GP, Heiman-Patterson TD. Cramp-fasciculation syndrome: a treatable hyperexcitable peripheral nerve disorder. *Neurology* 1991;41:1021–1024.
9. Vincent A, Beeson D, Lang B. Molecular targets for autoimmune and genetic disorders of neuromuscular transmission. *Eur J Biochem* 2000;267:6717–6728.
10. Storm JF. Potassium currents in hippocampal pyramidal cells. *Prog Brain Res* 1990;83:161–187.
11. Browne DL, Gancher ST, Nutt JG. Episodic ataxia/myokymia syndrome is associated with point mutations in the human potassium channel gene, KCNA1. *Nat Genet* 1994;8:136–140.
12. Lehmann-Horn F, Jurkat-Rott K. Voltage-gated ion channels and hereditary disease. *Physiol Rev* 1999;79:1317–1372.
13. Eunson LH, Rea R, Zuberi SM. Clinical, genetic, and expression studies of mutations in the potassium channel gene KCNA1 reveal new phenotypic variability. *Ann Neurol* 2000;48:647–656.
14. Kullmann DM. The neuronal channelopathies. *Brain* 2002;125:1177–1195.
15. Dedek K, Kunath B, Kananura C, Reuner U, Jentsch TJ, Steinlein OK. Myokymia and neonatal epilepsy caused by a mutation in the voltage sensor of the KCNQ2 K⁺ channel. *Proc Natl Acad Sci USA* 2001;98:12272–12277.
16. Delmas P, Brown DA. Pathways modulating neural KCNQ/M (Kv7) potassium channels. *Nat Rev Neurosci* 2005;6:850–862.
17. Ronen GM, Rosales TO, Connolly M, Anderson VE, Leppert M. Seizure characteristics in chromosome 20 benign familial neonatal convulsions. *Neurology* 1993;43:1355–1360.
18. Biervert C, Schroeder BC, Kubisch C. A potassium channel mutation in neonatal human epilepsy. *Science* 1998;279:403–406.
19. Charlier C, Singh NA, Ryan SG. A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. *Nat Genet* 1998;18:53–55.
20. Singh NA, Charlier C, Stauffer D. A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. *Nat Genet* 1998;18:25–29.
21. Jentsch TJ. Neuronal KCNQ potassium channels: physiology and role in disease. *Nat Rev Neurosci* 2000;1:21–30.
22. Singh NA, Westenskow P, Charlier C. KCNQ2 and KCNQ3 potassium channel genes in benign familial neonatal convulsions: expansion of the functional and mutation spectrum. *Brain* 2003;126:2726–2737.
23. Steinlein OK. Genetic mechanisms that underlie epilepsy. *Nat Rev Neurosci* 2004;5:400–408.
24. Lerche H, Weber YG, Jurkat-Rott K, Lehmann-Horn F. Ion channel defects in idiopathic epilepsies. *Curr Pharm Des* 2005;11:2737–2752.
25. Devaux JJ, Kleopa KA, Cooper EC, Scherer SS. KCNQ2 is a nodal K⁺ channel. *J Neurosci* 2004;24:1236–1244.
26. Lerche H, Biervert C, Alekov AK, et al. A reduced K⁺ current due to a novel mutation in KCNQ2 causes neonatal convulsions. *Ann Neurol* 1999;46:305–312.
27. Wuttke TV, Seeböhm G, Bail S, Maljevic S, Lerche H. The new anticonvulsant retigabine favors voltage-dependent opening of the Kv7.2 (KCNQ2) channel by binding to its activation gate. *Mol Pharmacol* 2005;67:1009–1017.
28. Wang HS, Pan Z, Shi W, et al. KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel. *Science* 1998;282:1890–1893.
29. Yang WP, Levesque PC, Little WA, et al. Functional expression of two KvLQT1-related potassium channels responsible for an inherited idiopathic epilepsy. *J Biol Chem* 1998;273:19419–19423.
30. Peretz A, Sheinin A, Yue C, et al. Pre- and postsynaptic activation of M-channels by a novel opener dampens neuronal firing and transmitter release. *J Neurophysiol* 2007;97:283–295.
31. Vervaeke K, Gu N, Agdestein C, Hu H, Storm JF. Kv7/KCNQ/M-channels in rat glutamatergic hippocampal axons and their role in regulation of excitability and transmitter release. *J Physiol* 2006;576:235–256.
32. MacKinnon R. Determination of the subunit stoichiometry of a voltage-activated potassium channel. *Nature* 1991;350:232–235.
33. Papazian DM. Potassium channels: some assembly required. *Neuron* 1999;23:7–10.
34. Tytgat J, Hess P. Evidence for cooperative interactions in potassium channel gating. *Nature* 1992;359:420–423.
35. Mannuzzo LM, Isacoff EY. Independence and cooperativity in rearrangements of a potassium channel voltage sensor revealed by single subunit fluorescence. *J Gen Physiol* 2000;115:257–268.
36. Ledwell JL, Aldrich RW. Mutations in the S4 region isolate the final voltage-dependent cooperative step in potassium channel activation. *J Gen Physiol* 1999;113:389–414.
37. Wuttke TV, Lerche H. Novel anticonvulsant drugs targeting voltage-dependent ion channels. *Expert Opin Investig Drugs* 2006;15:1167–1177.
38. Rundfeldt C. Characterization of the K⁺ channel opening effect of the anticonvulsant retigabine in PC12 cells. *Epilepsy Res* 1999;35:99–107.
39. Rundfeldt C, Netzer R. The novel anticonvulsant retigabine activates M-currents in Chinese hamster ovary-cells transfected with human KCNQ2/3 subunits. *Neurosci Lett* 2000;282:73–76.
40. Schenzer A, Friedrich T, Pusch M, Saftig P, Jentsch TJ, Grötzinger J, Schwake M. Molecular determinants of KCNQ (Kv7) K⁺ channel sensitivity to the anticonvulsant retigabine. *J Neurosci*. 2005;25:5051–5060.

Peripheral nerve hyperexcitability due to dominant-negative KCNQ2 mutations

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