

# Novel *KCNQ3* Mutation in a Large Family with Benign Familial Neonatal Epilepsy: A Rare Cause of Neonatal Seizures

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## Key Words

Familial neonatal convulsions · *KCNQ2* · *KCNQ3* ·  
Levetiracetam · Neonatal seizures · Potassium channels

## Abstract

Benign familial neonatal seizures (BFNS) present a rare familial epilepsy syndrome caused by genetic alterations in the voltage-gated potassium channels Kv7.2 and Kv7.3, encoded by *KCNQ2* and *KCNQ3*. While most BFNS families carry alterations in *KCNQ2*, mutations in *KCNQ3* appear to be less common. Here, we describe a family with 6 individuals presenting with neonatal focal and generalized seizures. Genetic testing revealed a novel *KCNQ3* variant, c.835G>T, cosegregating with seizures in 4 tested individuals. This variant results in a substitution of the highly conserved amino acid valine localized within the pore-forming transmembrane segment S5 (p.V279F). Functional investigations in *Xenopus laevis* oocytes revealed a loss of function, which supports p.V279F as a pathogenic mutation. When p.V279F was co-expressed with the wild-type (WT) Kv7.2 subunits, the resulting potassium currents were about 10-fold reduced com-

pared to the WT Kv7.3 and Kv7.2 coexpression. Genotype-phenotype correlation shows an incomplete penetrance of p.V279F. Response to antiepileptic treatment was variable, but evaluation of treatment response remained challenging due to the self-limiting character of the disease. The identification of the pathogenic variant helped to avoid unnecessary investigations in affected family members and allowed guided therapy.

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Benign familial neonatal epilepsy (BFNS1/2) is a rare inherited epilepsy disorder (OMIM 121200 and 121201) first described by Rett and Teubel [1964]. Mutations in the 2 genes *KCNQ2* in chromosome 20q and *KCNQ3* in chromosome 8q encoding the voltage-gated potassium channel subfamily KQT members 2 (Kv7.2) and 3 (Kv7.3) have been reported to result in BFNS [Biervert et al., 1998, Charlier et al., 1998, Singh et al., 1998]. *KCNQ2*, *KCNQ3*, and *KCNQ5* encode subunits of the widely expressed homo- and heterotetrameric M-channels, yielding the slowly activating and deactivating potassium M-current,

which can be blocked by muscarinic agonists [Maljevic and Lerche, 2014]. In >95% of all cases, BFNS can be attributed to mutations in *KCNQ2*. Only a few mutations in *KCNQ3* have so far been associated with this disorder and associated electrophysiological data is scarce (HGMD, <http://www.hgmd.cf.ac.uk/>) [Singh et al., 2003, Zara et al., 2013, Maljevic and Lerche, 2014].

Our study aimed to report phenotypic data on members of a BFNS family carrying a novel missense mutation in *KCNQ3* and to investigate the impact of the mutation on channel function.

## Methods

### Genetic Testing

Genomic DNA of the index patient (IP) and available family members was extracted from peripheral blood lymphocytes. The coding exons of *KCNQ2* (accession number hg19: NM 172108.3) and *KCNQ3* (accession number hg19: NM 004519.2) were sequenced by conventional Sanger sequencing. Deletion/duplication screening was performed by MLPA. Predictions of the functional impact of identified coding variants were assessed by different *in silico* analysis programs (PolyPhen2, <http://www.genetics.bwh.harvard.edu/pph2> and MutationTaster, <http://www.mutationtaster.org>). Variants were compared to 60,706 controls of the ExAC browser (<http://exac.broadinstitute.org/>) and in-house data. Conservation of mutated positions was evaluated using sequence alignment of different species and different human Kv-channels.

### Functional Studies

#### Mutagenesis and RNA Preparation

The Quick Change kit (Stratagene, La Jolla, Calif., USA) was used to engineer the missense mutation in the *KCNQ3* cDNA inserted into the pTLN vector. The mutation was confirmed and additional mutations excluded by Sanger sequencing. Primers are available upon request. cRNA was prepared using the SP6 message mMachin kit from Ambion (Agilent Technologies, Santa Clara, Calif., USA).

#### Oocyte Preparation and Injection

Oocytes were obtained from the Institute of Physiology I, Tübingen and prepared as previously described [Orhan et al., 2014]. The concentrations of Kv7.2 and Kv7.3 mutant and wild-type (WT) cRNAs were adjusted to the same value and cRNA mixtures in appropriate ratios injected in parallel using Robooinject® (Multi Channel Systems, Reutlingen, Germany). The volume of injected cRNA was kept constant (50 nl).

#### Automated Oocyte 2-Microelectrode Voltage Clamp

Potassium currents in oocytes were recorded 2–3 days after injection using Roboocyte2® (Multi Channel Systems, Reutlingen, Germany). The intracellular glass microelectrodes had a resistance of 0.3–1 MΩ when filled with 1 M KCl/ 1.5 M KAc. The bath solution was ND96 (in mM: 93.5 NaCl, 2 KCl, 1.8 CaCl<sub>2</sub>, 2 MgCl<sub>2</sub>, and 5 Hepes; pH 7.5). Currents were sampled at 1 kHz. To analyze channel activation, we used 2-second long depolarizing steps ( $\Delta 10$

mV) from a holding potential of –80 to +40 mV, followed by a step to –30 mV for 0.5 s to analyze the tail currents [Orhan et al., 2014].

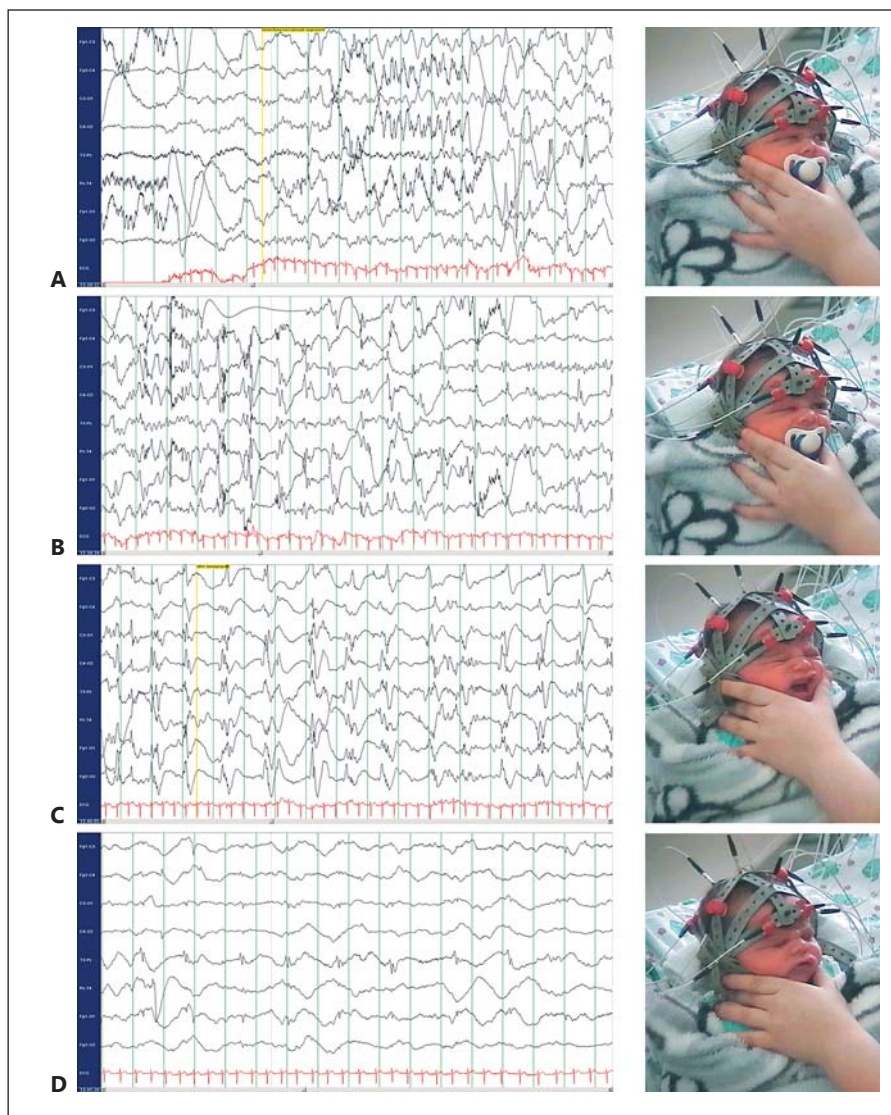
### Data Analysis

Voltage clamp recordings were analyzed using Roboocyte2+ (Multi Channel Systems), Clampfit (pClamp 8.2, Axon Instruments, Union City, Calif., USA), Microsoft Excel (Microsoft, Redmond, Wash., USA), and GraphPad Prism (GraphPad Software, La Jolla, Calif., USA). Maximum current amplitudes were compared at the end of a 2-second test pulse to +40 mV. The voltage dependence of channel activation was derived from tail current amplitudes recorded at –30 mV. A Boltzmann function was fit to the current-voltage relationships,  $I(V) = I_{max}/(1 + \exp[(V - V_{0.5})/k]) + C$ , where  $I_{max}$  is the maximum tail current amplitude at test potential  $V$ ,  $V_{0.5}$  the half-maximal activation potential,  $k$ , a slope factor reflecting characteristics of voltage-dependent channel gating and  $C$ , a constant. All data are shown as mean values  $\pm$  SEM. Statistical significance was evaluated using one-way ANOVA with Dunnett's post hoc test. Multiple comparisons were performed to Kv7.2/Kv7.3 channels as a control (GraphPad Prism).

## Results

### Clinical Features

The IP (IV.2), a 6-day-old eutrophic boy, born at term, was admitted to hospital because of a focal clonic, secondarily generalized tonic-clonic seizure with cyanosis lasting 1 min. Repetitive focal clonic seizures, involving legs and arms asymmetrically, had been observed from the day before. After admission, frequent generalized seizures with oxygen desaturation to 40% were observed, necessitating intensive care unit monitoring. Extensive investigations including infectiological, metabolic screenings (electrolytes, glucose, ammonia, acylcarnitines, very long-chain fatty acids, cerebrospinal fluid parameters, lactate, pterines, neurotransmitter, amino acids, pipercolic acid, urinary organic acids, sulfite test, and creatine metabolism) as well as brain MRI on postnatal day 6 (d6) gave normal results. Electroencephalography (EEG) repeatedly displayed focal epileptic discharges and clonic seizures of focal origin (fig. 1). Up to 5 seizures per day occurred until d20 with only 1 seizure on d18 and d19. After trials with pyridoxine and pyridoxal-phosphate, levetiracetam (LEV) was added on d8 with increasing doses reaching 65 mg/kg body weight on d20. The patient was discharged in good clinical condition on d25. Treatment with LEV was successfully discontinued after 8 months of age. The IP is now 4 years old and shows an age-appropriate development. Two first cousins of the IP (IV.4 and IV.5) were identified and treated after the identification of the pathogenic variant in this family. Both cousins received only basic investigations to rule out other causes of neonatal seizures. Pro-

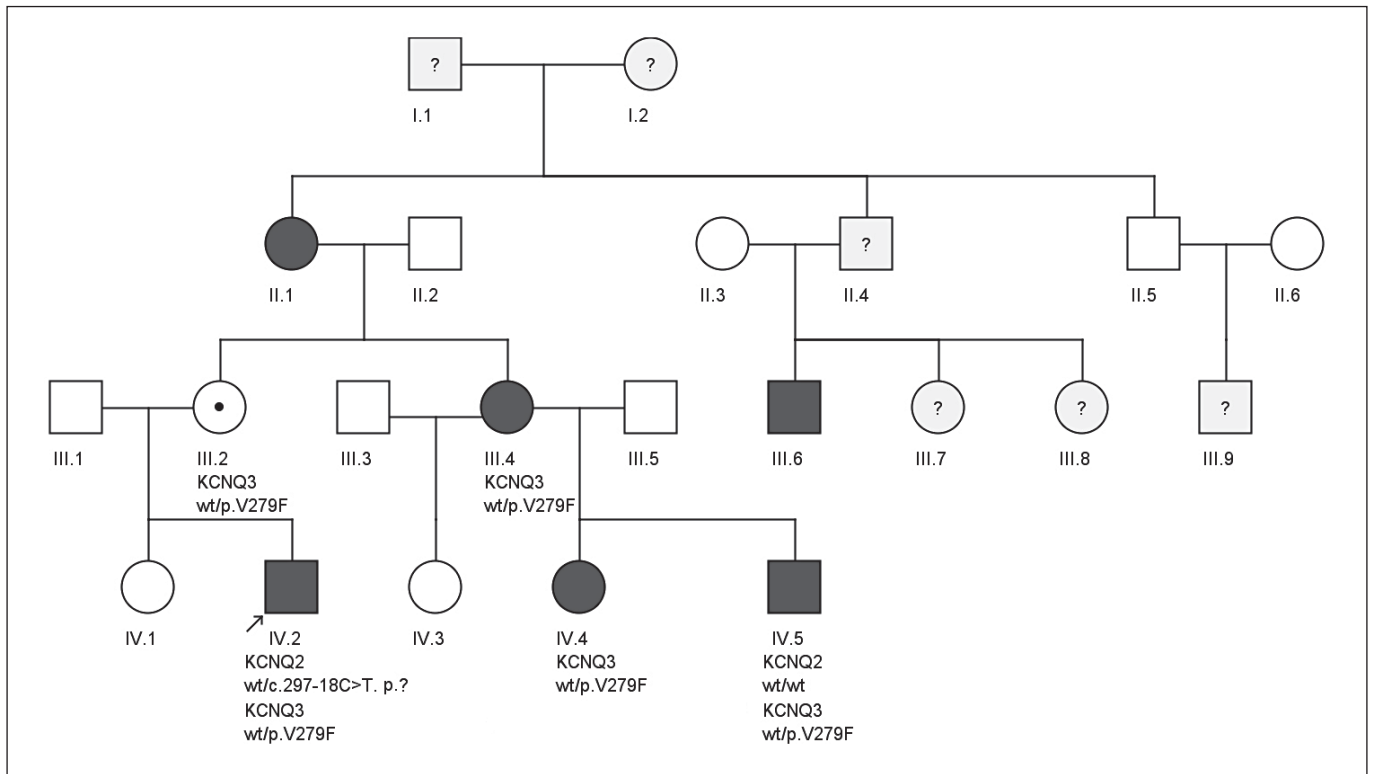


**Fig. 1.** Ictal Video-EEG recording of the IP on postnatal d7, documenting right hemispheric onset of a focal seizure (A) with upward gaze to the left side, hemifacial twitching (B) and clonus of the left hand (not seen). Secondly generalized rhythmic spike-wave activity (C), clinically accompanied by apnea and cyanosis is followed by generalized slowing of the background activity (D).

longed cyanotic seizures prompted anticonvulsant treatment in both. While IV.4 became seizure free after introduction of LEV on d12, seizures persisted despite LEV treatment (up to 85 mg/kg/d) in patient IV.5. He eventually became seizure free after switching to oxcarbazepine (OXC) after d10. The pedigree analysis (fig. 2) of the family revealed self-limited cyanotic neonatal seizures that prompted hospitalization in 3 more relatives, starting between d2 and d4. None of the affected individuals displayed seizures after the first month of life, independent of anticonvulsant treatment, and all showed a normal psychomotor development later in life. Clinical information is provided in table 1 for all affected family members where data was available.

#### Genetic Testing

In the IP, we detected the intronic *KCNQ2* variant c.297-18C>T and the *KCNQ3* missense variant c.835G>T, p.V279F. *KCNQ2* c.297-18C>T is repeatedly seen in controls in the ExAC database (56/60.706), whereas *KCNQ3* p.V279F is absent. Standard in silico prediction tools (PolyPhen2 and MutationTaster) predicted *KCNQ2* c.297-18C>T as benign and *KCNQ3* p.V279F as likely pathogenic with a very high confidence score (>0.999). Overall 6 affected individuals and 2 unaffected obligate carriers were identified within this family. Further segregation analysis confirmed the presence of the *KCNQ3* p.V279F in 4 more affected individuals and obligate carriers while *KCNQ2* c.297-18C>T did not



**Fig. 2.** Pedigree of the family with BFNS. Neonatal convulsions were reported in 6 individuals. The mutation *KCNQ3* p.V279F cosegregated with neonatal seizures in all tested individuals and obligate carriers.

cosegregate with the neonatal seizures in patient IV.5 (fig. 2).

#### Functional Analysis

*KCNQ3* p.V279F replaces the highly conserved amino acid valine at position 279 within the pore-forming transmembrane segment S5 of Kv7.3 channel (fig. 3A, B). To study the functional impact of the mutation we used *Xenopus laevis* expression system and automated 2-microelectrode voltage clamp technique. In contrast to Kv7.2, homomeric Kv7.3 channels seem to be unstable in heterologous expression systems, which is why we could not examine them separately. Therefore, *KCNQ2* cRNA was injected alone or co-injected with the cRNA encoding WT or mutant *KCNQ3*, and the resulting potassium currents were investigated. Current amplitudes, analyzed at the end of the 2-second depolarizing pulse to +40 mV, were normalized to the mean value obtained for the WT Kv7.2/Kv7.3 heteromers recorded on the same day. As expected, coexpression of Kv7.2 and Kv7.3 WT yielded about 15-fold increased currents compared to the WT

Kv7.2. However, when p.V279F was coexpressed with Kv7.2 WT, the resulting current reached only ~12% of the control Kv7.2/Kv7.3 currents, indicating a clear loss of function ( $p < 0.0001$ , one-way ANOVA, fig. 3C). We further observed tentative reduction, but no significant difference compared to the WT Kv7.2/Kv7.3 current amplitudes, when we coexpressed Kv7.2 with Kv7.3 WT and the V279F mutation in a 2:1:1 ratio, mimicking the putative proportion of mutant and WT subunits in an affected individual. Moreover, the voltage dependence of activation of the Kv7.2/Kv7.3/p.V279F was not changed compared to the WT Kv7.2/Kv7.3 channels (fig. 3D).

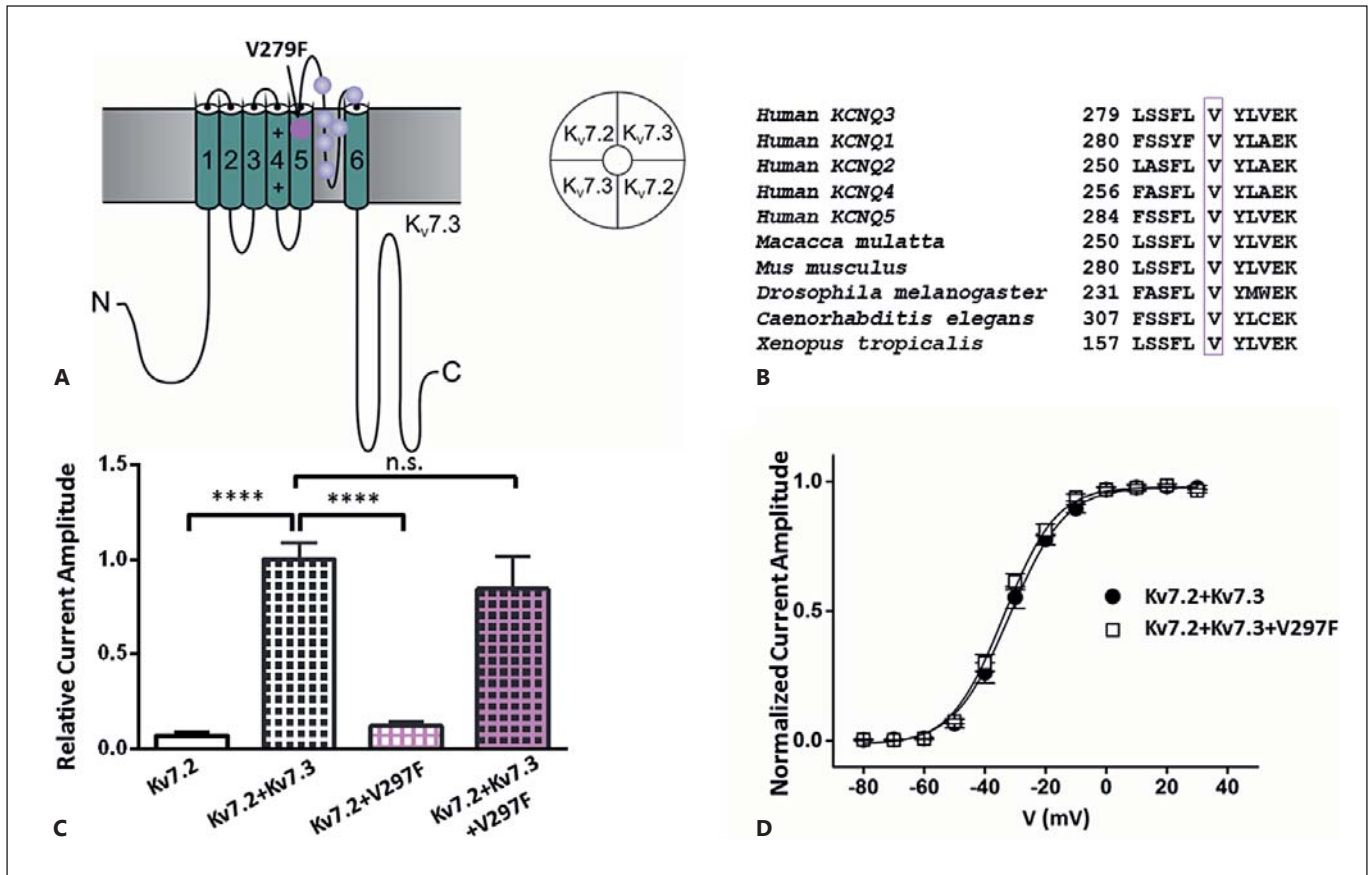
#### Discussion

We identified 6 affected individuals in 3 generations of a family with neonatal convulsions starting between d2 and d6 (fig. 2). Seizures were mainly focal clonic seizures occurring during wake or sleep over a period of a few days up to 2 weeks. Additional generalized seizures with cya-

**Table 1.** Clinical details of family members with reported neonatal seizures

Patients	IV.2 (IP)	IV.4	IV.5	III.4	II.1	III.6
Current age, years	4	2	1	31	55	19
Mutation	KCNQ3 c.835G>T (het) p.V279F KCNQ2 c.297-18C>T p.?	KCNQ3 c.835G>T (het) p.V279F	KCNQ3 c.835G>T (het) p.V279F KCNQ2 wild type	KCNQ3 c.835G>T (het) p.V279F	not done	not done
Sex	M	F	M	F	F	M
Exam at birth (length, weight, OFC)	48 cm, 3,130 g, 34.5 cm, Apgar 10/10/10, pH 7.28	2,725 g, 47 cm, 39 cm	3,160 g, 47 cm, 35 cm, Apgar 9/10/10	3,000 g, 49 cm, 33 cm, Apgar 9/10/10	not known	not known
Gestational age, weeks	39+2	39+2	39+0	39+3	not known	not known
Seizure onset (day of life)	d5	d6	d5	d3	during first week	during first week
Seizure type at onset	focal clonic, secondary GTCS with cyanosis, multiple per day	focal clonic, secondary GTCS with cyanosis, multiple per day	focal clonic, secondary GTCS with cyanosis, multiple per day	tonic and focal clonic, GTCS with cyanosis, multiple per day	cyanotic spells	cyanotic spells
Seizure cessation (day of life)	d20	d12	d10	d4	not available	not available
Treatment and outcome	unsuccessful trials with pyridoxine, pyridoxal- phosphate, folinic acid, LEV after d9, slowly increasing up to 65 mg/kg on d20 (no further seizures after d20)	LEV after d12, up to 70 mg/kg (no further seizures after first dose)	LEV after d6, up to 85 mg/kg on d8 (still up to 4 seizures per day), OXC after d10, up to 20 mg/kg (no further seizures after first dose)	no treatment	no treatment, spells prompted cardiological monitoring and investigations	not available
Associated movement disorder	not reported	not reported	not reported	not reported	not reported	not reported
Seizure outcome	seizure free	single febrile seizure at 5 months	seizure free	putative seizure at 12 years	seizure free	seizure free
EEG at onset	focal sharp wave on ictal and interictal EEG, normal background activity	focal sharp wave on ictal EEG, normal interictal EEG, normal background activity	normal interictal EEG, normal background activity	normal interictal EEG, normal background activity	not known	not known
EEG at follow-up	normal	at 6 weeks, focal sharp waves on interictal EEG, normal EEG after 5 months	normal	normal	not known	not known
Developmental outcome	normal at 4 years	normal at 2 years	normal	normal	normal	normal
Brain imaging	normal MRI at d6	normal ultrasound at d6	normal ultrasound at d6	not done	not done	not done
Additional investigations	normal results for tandem- MS, urinary organic acids, creatine metabolism; CSF analysis incl. glucose quotient, neurotransmitter, pterines, sulfite test	no additional investigations	no additional investigations	CSF analysis	not known	not known

CSF = Cerebrospinal fluid; GTCS = generalized tonic-clonic seizures; OFC = occipitofrontal circumference; tandem-MS = tandem mass spectrometry.



**Fig. 3.** Functional impact of the Kv7.3 mutation V279F expressed in *X. laevis* oocytes. **A** Kv7.3 channel subunit comprises intracellular N- and C-terminal domains and 6 transmembrane segments, with a pore-forming S5–S6 region. p.V279F predicted localization is within the S5 segment. Previously reported BFNS mutations in the *KCNQ3* are located in the pore-forming region [modified after Maljevic and Lerche, 2014]. **B** p.V279F and the surrounding amino acids show evolutionary conservation. **C** Expression of WT Kv7.2 and WT Kv7.2 with WT Kv7.3 and/or V279F. Mean  $\pm$  SEM of relative current amplitudes analyzed at +40 mV and normalized to

the mean of the Kv7.2 + Kv7.3 currents recorded on the same day were as follows:  $0.07 \pm 0.01$  for Kv7.2;  $1.0 \pm 0.1$  for Kv7.2 + Kv7.3;  $0.11 \pm 0.02$  for Kv7.2 + V279F, and  $0.84 \pm 0.07$  for Kv7.2 + V279F + Kv7.3 (2:1:1);  $n = 7-31$ ; \*\*\*\*  $p < 0.0001$  with one-way ANOVA and Dunnett's post hoc test. **D** Current-voltage relationships of Kv7.2 + Kv7.3 and Kv7.2 + Kv7.3 + V279F (2:1:1) determined from tail current amplitudes. Lines represent fits of a Boltzmann function.  $V_{0.5}$  was  $-32 \pm 2$  ( $n = 8$ ) for Kv7.2 + Kv7.3 and  $-34 \pm 1$  ( $n = 9$ ) for Kv7.2 + Kv7.3 + V279F (2:1:1).

nosis lasting several minutes prompted treatment in 3 patients (IV.2, IV.4, and IV.5). One child showed a fever-associated seizure after withdrawal of treatment (IV.4), and one individual had another putative seizure during adolescence (III.4), which is in line with reported seizures later in life in members of affected families [Singh et al., 2003; Neubauer et al., 2008].

In the IP, we identified 2 novel variants of unknown significance in *KCNQ2* and *KCNQ3*. The *KCNQ2* variant, lying outside the coding sequence, was not predicted to alter splicing and, given its occurrence in control cohorts, is likely to be a benign polymorphism. Further-

more, it did not cosegregate with the disease in the family. In contrast, the *KCNQ3* variant is predicted to be pathogenic and is absent in the ExAc browser. The variant cosegregated with neonatal seizures in all family members that were available for genetic analysis (fig. 2). As 6 out of 8 known or obligate carriers showed self-limited neonatal seizures, genotype-phenotype analysis of p.V279F suggests incomplete penetrance.

Whereas mutations in *KCNQ2* have been identified in the majority of BFNS cases, the number of associated *KCNQ3* variants is much smaller: 8 in HGMD or 14 in the *KCNQ2/3* database (<http://www.rikee.org>). Consequent-

ly, previous functional studies have mainly focused on mutations in *KCNQ2* and data on functional consequences of mutated Kv7.3 channels is rare with nonconclusive results [Uehara et al., 2008; Soldovieri et al., 2014].

We studied the impact of p.V279F in *X. laevis* oocytes by coexpressing it with the WT Kv7.2 subunits. The coexpression of Kv7.2 with Kv7.3 results in a 10- to 15-fold increase of potassium currents recorded in oocytes, probably due to the increase in the surface expression of the Kv7.2 channels [reviewed in Maljevic and Lerche, 2014]. However, currents obtained from the coexpression of p.V279F with Kv7.2 yielded only about 10% of the Kv7.2/Kv7.3 WT current, suggesting that the mutant subunit is either absent, not able to form heteromeric channels, or produces trafficking- or functionally defective heterotetramers with Kv7.2 subunits. Given its position within the pore-forming S5–S6 region of the Kv7.3 channel, the last 2 explanations seem more plausible. If the mutation affects folding of the channel protein, it may not pass the cellular control mechanisms and reach the surface membrane. Alternatively, the mutation may affect channel gating [Maljevic and Lerche, 2014].

In the coexpression experiments supporting the expected expression of mutant and WT subunits in a mutation carrier (Kv7.2:Kv7.3:p.V279F in a 2:1:1 ratio), the observed current reduction was statistically not significant compared to the WT Kv7.2/Kv7.3 coexpression and reached about 15%, instead of the expected 25% associated with a haploinsufficiency pathomechanism due to a loss of function of 1 of the 4 alleles. The observed tentative reduction of the heteromeric Kv7.2/Kv7.3 currents has been previously seen for some Kv7.2 BFNS mutations in *X. laevis* oocytes [Orhan et al., 2014] and may indicate that the pathophysiological mechanisms in vivo involve other factors not present in our expression system.

So far, haploinsufficiency of Kv7 is known to result in BFNS with only a small number of reported dominant negative missense mutations in *KCNQ2* and *KCNQ3* [Uehara et al., 2008; Maljevic and Lerche, 2014]. A dominant negative effect indicates that the presence of the mutant impairs the function of the remaining WT subunits, causing further, more pronounced reduction of potassium currents. The majority of dominant negative mutations in *KCNQ2* result in more severe phenotypes with associated neurological disorders such as peripheral nerve hyperexcitability or less favorable seizure outcome and intellectual disability, known as *KCNQ2* encephalopathy [Wuttke et al., 2007; Weckhuysen et al., 2013; Orhan et al., 2014]. Interestingly, a recent study also reported mutations in the voltage sensor resulting in a gain of

Kv7.2 channel function associated with a severe epilepsy phenotype [Miceli et al., 2015a]. Only a few reports involved functional characterization of *KCNQ3* mutations [Uehara et al., 2008; Suguira et al., 2009; Soldovieri et al., 2014; Miceli et al., 2015a, b]. The results varied from mere reduction in current amplitudes [Suguira et al., 2009; Soldovieri et al., 2014] over a dominant negative effect associated with BFNS [Uehara et al., 2008] or with a more severe phenotype with intellectual disability [Miceli et al., 2015b], to the mentioned gain of function in severe epilepsy [Miceli et al., 2015a].

Interestingly, no deletion or truncating mutation has been reported in *KCNQ3* to date. In line with this finding, a knockout mouse model is viable, and the lack of *KCNQ3* does not produce a specific phenotype [Tzingounis and Nicoll, 2008]. In contrast, homozygous knockin mice carrying a *KCNQ3* BFNS-causing mutation develop spontaneous seizures [Singh et al., 2008]. Furthermore, known *KCNQ3* mutations seem to cluster within the pore region of the channel (fig. 3A) critical for channel gating. Altogether, this may argue that unlike the *KCNQ2*, which can cause disease by both haploinsufficiency as well as dominant negative mechanism, the *KCNQ3* pathogenicity is based on the impact of single amino acid exchanges on the function of affected channels. Due to the low expression of the Kv7.3 channel in heterologous expression systems, this impact is usually assessed using the Kv7.2/Kv7.3 heterotetramers. However, also other mechanisms, involving the homomeric Kv7.3 or heteromeric Kv7.5/Kv7.3 channels may play a role in epileptogenesis. Therefore, further identification of *KCNQ3* mutations and functional characterization of their consequences in neuronal environment are necessary to elucidate the role of *KCNQ3* in BFNS.

Three individuals were treated for repetitive neonatal seizures. As many seizures were accompanied by apnea and significant desaturation, treating physicians refrained from a conservative treatment and applied antiepileptic drugs (AED). Depicting the outcome of AED treatment in the family members remained challenging. While 2 patients became seizure free during treatment with LEV after d12 (IV.4) and d20 (IV.2), respectively, patient IV.5 still displayed seizures after introduction of LEV from d6 to d10. He eventually became free of seizures with introduction of OXC after d10. OXC is of specific benefit in *KCNQ2*-related epilepsy/encephalopathy [Weckhuysen et al., 2013]. Retigabine, a novel AED, specifically activating Kv7 channels by shifting the voltage sensitivity seems to be an ideal candidate compound for *KCNQ2/3*-associated seizure disorders [Rundfeldt and Netzer, 2000].

However, taking into account both the benign course of disease and the self-limiting character, retigabine and other novel AED with potential adverse drug reactions seem less appropriate in the setting of benign neonatal seizures.

In conclusion, we report on the novel mutation *KCNQ3* p.V279F as the cause of neonatal seizures in a family. Functional studies add further evidence for the pathogenicity of p.V279F, demonstrating a loss of channel function. Our study highlights the importance of genetic screening in families with neonatal seizures. The knowledge of a familial mutation can help families and caring neonatologists to avoid unnecessary invasive diagnostic and therapeutic attempts. Further studies on *KCNQ3* mutations will help to elucidate the role of *KCNQ3* in epileptic disorders.

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## Statement of Ethics

The use of animals and all experimental procedures were approved by local authorities (Regierungspräsidium Tübingen, Tübingen, Germany).

## Disclosure Statement

The authors have no conflicts of interest to declare.