Quinidine in the Treatment of KCNT1-Positive Epilepsies

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Abstract

We report 2 patients with drug-resistant epilepsy caused by *KCNT1* mutations who were treated with quinidine. Both mutations manifested gain of function in vitro, showing increased current

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that was reduced by quinidine. One, who had epilepsy of infancy with migrating focal seizures, had 80% reduction in seizure frequency as recorded in seizure diaries, and partially validated by objective seizure evaluation on EEG. The other, who had a novel phenotype, with severe nocturnal focal and secondary generalized seizures starting in early childhood with developmental regression, did not improve. Although quinidine represents an encouraging opportunity for therapeutic benefits, our experience suggests caution in its application and supports the need to identify more targeted drugs for *KCNT1* epilepsies.

K*CNT1* mutations have recently been implicated in a range of epilepsy syndromes including severe autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE)[1,2] and epilepsy of infancy with migrating focal seizures (EIMFS).[3,4] Mutations result in KCNT1 channel gain of function. This gain of function, the magnitude of which correlates with the clinical severity, can be reduced by quinidine in vitro.[5] A recent case report described improvement in seizure control with quinidine in a patient with *KCNT1*-EIMFS.[6] Here, we report 2 patients with different epilepsy phenotypes caused by *KCNT1* mutations with different responses to quinidine therapy. We also analyze the cases for additional factors that might have resulted in variable therapeutic response and discuss how this could help development of future tailored therapies for such disorders. This work was approved by our institutional review board.

Case Reports

Patient 1

Patient 1 is an 11-year-old female who had normal growth and development until the age of 18 months, when she developed nighttime "gagging" spells initially attributed to "allergies," followed by nocturnal generalized tonic–clonic seizures as of the age of 2.5 years. Initial electroencephalogram (EEG) was normal, but long-term monitoring demonstrated that the gagging spells were nocturnal focal seizures arising from the right hemisphere. Her nocturnal tonic–clonic seizures rapidly progressed in frequency to 10 to 15 per night. Over the following year, she started to regress and eventually lost all of her expressive speech and fine motor skills; at age 5 years, she was still ambulatory, but very ataxic. In the following year, she became nonambulatory and noncommunicative and had to be fed by Gtube. EEG showed multifocal discharges emanating from the right and left posterior head regions and right temporal region. Frequent prolonged bursts of generalized poly-spike and wave discharges were also noted in sleep. Extensive metabolic and genetic investigations were negative. Whole exome sequencing (WES) revealed a heterozygous de novo *KCNT1* mutation (NM_020822.1:c.2386T>C; p.[Tyr796His], Y796H). This exact variant has been previously reported in a family with ADNFLE.[2] Functional testing in vitro showed that the Y796H mutation resulted in channels with a significantly greater magnitude of peak current as compared to the wild-type (WT) channels (Fig 1A, C). However, this effect was on the milder side of the functional spectrum when compared to K629N, the mutation identified in Patient 2, and R428Q, the mutation reported in the recently published case.[6] Quinidine (300 μM) produced significant inhibition (36.1±6.7%) of the Y796H channel, bringing current levels down toward WT control values (see Fig 1B, D). When seen for evaluation for quinidine therapy, the patient was having multiple nocturnal mostly tonic, rarely tonic–

clonic, seizures/ day. She had diffuse decrease in tone. Reflexes and plantar responses were normal. Twelve antiepileptic medications and the ketogenic diet had failed. EEG showed a diffusely slow background with bilateral frontal spikes interictally, and during her recorded generalized tonic seizures an electrodecremental response. Magnetic resonance imaging (MRI) showed global atrophy. She was admitted and started on quinidine 11mg/kg/day in 3 divided doses achieved gradually over 3 days. Over the following month, her mean quinidine serum level was 0.6 μg/ml (0.4 and 0.8 μg/ml; therapeutic for cardiac effects: 2–5 μg/ml). She was readmitted, and the dose was increased over 3 days to 40mg/kg/day in 3 divided doses. Her mean level rose during the following 2 months to 2.4 μg/ml (2.4, 2.1, 3.3, and 1.7 μg/ml). She was then readmitted again, and the was dose increased over 3 days to 54.2 μg/kg/day. The level however did not rise over the next month; mean was 1.7 μg/ml (2, 1.3 μg/ml). The dose was then decreased and maintained during the following month to 34mg/kg/ day. Seizure frequency showed no improvement from baseline (Fig 2A). During the first hospitalization the patient had, before quinidine was started during the first 24 hours of admission, 10 seizures which were detected by video EEG monitoring (10 seizures/24 hours). She then had 52 seizures over the next 5 days while quinidine was being started and increased (13 seizures/24 hours). During the second hospitalization she had 23 seizures over 4 days (5.45 seizures/24 hours), and during the third hospitalization she had 39 seizures over 5 days (7.8 seizures/24 hours). EEGs done during each admission did not show any significant changes. Developmentally she was noted to be minimally more alert and interactive. At the 54.2 μg/ kg/day dose, some prolongation of the QT interval was observed, which precluded further increases.

Patient 2

Patient 2 is a 3-year-old male who was hospitalized as a neonate for 4 months for episodes of multiple daily focal seizures and status epilepticus that required pentobarbital coma. The electroclinical picture was consistent with EIMFS. Seizures usually lasted 10 seconds to 3 minutes and consisted of: (1) unilateral upper and lower extremity clonic activity with adversive head turning and nystagmus, (2) eye deviation, (3) generalized tonic stiffening, and (4) lip smacking with asymmetric jerking of the extremities. EEG showed diffuse delta slowing with very frequent multifocal spikes and electrographic seizures occurring as frequently as every 2 to 10 minutes, lasting an average of about 30 seconds and emanating independently from C3, C4, T3, T4, O1, or O2. Extensive metabolic workup was negative; ammonia, lactate, pyruvate, amino acids, acylcarnitine profile, creatine, guanidinoacetate, biotinidase, pipecolic acid, alpha aminoadipic acid semialdehyde (serum), organic acids, sulfocysteine, succinyl purines (urine), glucose, protein, amino acids, lactate, neurotransmitter metabolites, neopterin, biopterin, 5-methyl tetrahydrofolate, folate, homocysteine, and pyridoxal-5-phospahate (cerebral spinal fluid [CSF]) were all normal. He also had negative workup including muscle biopsy for mitochondrial diseases. DNA testing for known epilepsy syndromes was unremarkable. He continued to have multiple seizures per day, which evolved to tonic seizures over the following months. At 3 years, WES revealed a de novo *KCNT1* mutation (NM_020822.2:c.1887G>C; p.[Lys629Asn], K629N). Functional testing in vitro showed that K629N also resulted in a gain-of-function phenotype, the magnitude of which was quite striking when compared to both WT and Y796H, but not R428Q (see Fig 1A, C). Quinidine (300 μM) was less effective in inhibiting K629N channel

current (11.5±1.7% inhibition) as compared to WT (42.7±2.7% inhibition), Y796H (36.1±6.7% inhibition), or R428Q (25.8±3.2% inhibition; see Fig 1B, D). When evaluated for quinidine therapy, he was having multiple daily seizures. Eight antiepileptic medications and the ketogenic diet had failed. His examination showed microcephaly, central hypotonia, extremity hypertonia, and hyperreflexia with bilateral Babinski reflex. EEG showed interictal multifocal spikes and ictal electrodecremental fast beta rhythms and multifocal subclinical electrographic seizures. MRI showed diffuse atrophy. He was admitted and started on 12mg/kg/day of quinidine in 3 divided doses, which was gradually increased over 4 days to 22.6mg/kg/ day in 3 divided doses. Comedications were kept at the same doses and the levels remained within the ranges established before quinidine therapy. Over the next 2 months, quinidine serum level was 0.3 μg/ml. QT interval did not show any significant changes, so the patient was readmitted and the dose was increased gradually over 4 days to 34.4mg/kg/day divided into 3 doses. During the first hospitalization before quinidine was started, the patient had during the first 12 hours of admission 8 seizures detected by video EEG monitoring (16 seizures/24 hours). He then had 4 seizures over the next 2 days (2 seizures/24 hour) while quinidine was being started and increased. During the second hospitalization he had 6 seizures over 48 hours (3 seizures/24 hours), and during the third admission he had 33 seizures over 4 days (8.25 seizures/ 24 hours). EEGs done during each admission did not show any significant changes. Over the next month, the mean level was 0.77 μg/ml (1.1, 0.6, and 0.6 μg/ml). Seizure frequency decreased by 80% as shown in Figure 2B. Developmentally he was more alert and more interactive.

Discussion

Using the approach of a translational paradigm for in vitro studies to inform novel therapies for epilepsy, we treated 2 patients with quinidine.[7] Case 1, who had a novel phenotype resembling severe nocturnal frontal lobe epilepsy, albeit with additional posterior and generalized EEG changes, which could be considered intermediate between EIMFS and typical nocturnal frontal lobe epilepsy, demonstrated no response, whereas Case 2, who had neonatal onset EIMFS, had a clinically meaningful improvement.

Earlier work suggested possible poor accumulation of quinidine in the CSF of human subjects (4–37%, average±16%).[8] However, work in rodents showed it can achieve concentrations higher than those in the serum.[9] CSF access is also supported by the antiepileptic effects of quinidine in a number of animal models.[10,11] These have included focal penicillin, electrically induced seizures, and bupivacaine-induced generalized seizures. These findings, and the effects of quinidine on KCNT1 channel dysfunction, supported using it in our 2 patients. Quinidine metabolism is enhanced by enzyme inducers, which is probably the reason why our second patient had low levels, as he was on phenobarbital.[12] Although quinidine has been reported to interact with a number of medications,[13–16] it has not been reported to specifically affect the metabolism of clobazam, levetiracetam, or phenobarbital. The levels of these medications remained within the ranges documented before quinidine was initiated. This argues in favor of a true response to quinidine in Patient 2. Quinidine can cause prolongation of the QT interval; thus, both patients underwent continuous electrocardiographic (ECG) monitoring throughout their inpatient admissions

during which the quinidine dosages were being increased, and also underwent routine ECGs and Holter monitoring on a monthly basis during the outpatient follow-up visits.

The available data are too limited to reach firm conclusions about any possible correlation between specific mutations and the response to quinidine. Our first patient had a severe presentation and did not respond to quinidine although she carries the Y796H mutation that was previously found in a family with ADNFLE, and that is on the more mild side of the in vitro functional spectrum. Our second patient had a favorable response albeit less so than the patient reported by Bearden et al,[6] although the mutation he carries is the least sensitive mutation to quinidine in vitro. The patient of Bearden et al[6] showed the best response, and carried the mutation that was the most sensitive to quinidine (see Fig 1D). This raises the possibility that the in vitro effect of quinidine may predict, in some but not in all patients, clinical response. The current limited and variable data, however, both in terms of in vitro effects and clinical responses makes it impossible to draw any clear conclusions about in vitro responses and clinical responses. Thus, our data justifies a need for further studies of quinidine in KCNT1-related epilepsies, and illustrates that in vitro work can guide development and investigation of clinical therapies. Our cases also demonstrate that clinical response can vary. This cautions against wide and indiscriminate use of quinidine in patients outside carefully designed protocols and provides information that can help in the design of future controlled studies. Specifically, our data, combined with the previous work, make the important practical point that there is some phenotype– genotype correlation and that anyone considering quinidine therapy should first assess the quinidine response of the specific mutation found in the patient. We feel that this work will directly contribute to the more careful application of quinidine going forward, which is becoming a pressing concern as we learn of an increasing number of patients with mutations of unknown significance in *KCNT1* being considered for quinidine therapy. We also note that making the case for this functional assessment before targeted treatment is initiated would have the additional benefit of ensuring that quinidine is not initiated when there is a benign variant, or a mutation that results in loss rather than gain of function, which is also becoming an increasing concern.

Differences in response to quinidine may relate to: (1) interindividual variability in crossing the blood–brain barrier; (2) differences in the efflux of quinidine from the brain;[9] (3) the developmental window in which quinidine is given; (4) injury resulting from previous severe seizures; (5) limitations on dosage due to quinidine prolongation of QT interval; or (6) additional unrecognized pathophysiological factors that lead to different phenotypes, as may be the case in our Patient 1.

In conclusion, the therapeutic effects of quinidine in *KCNT1* positive epilepsy remain largely unknown. The question of how much quinidine may help, if it all, and in which specific types of *KCNT1* positive epilepsy and for which mutations can only be resolved by more detailed clinical evaluation, ideally using objective measurements of seizures since we may expect responses to be inflated by expectation in the absence of objective measures. Ideally, evaluation of quinidine in *KCNT1* animal models would guide such clinical evaluations. Although the current limited data do not appear to support the idea of substantial clinical benefit of quinidine, quinidine does clearly illustrate a new potential

paradigm for the development and clinical evaluation of genetically targeted therapies in epilepsy.

Acknowledgments

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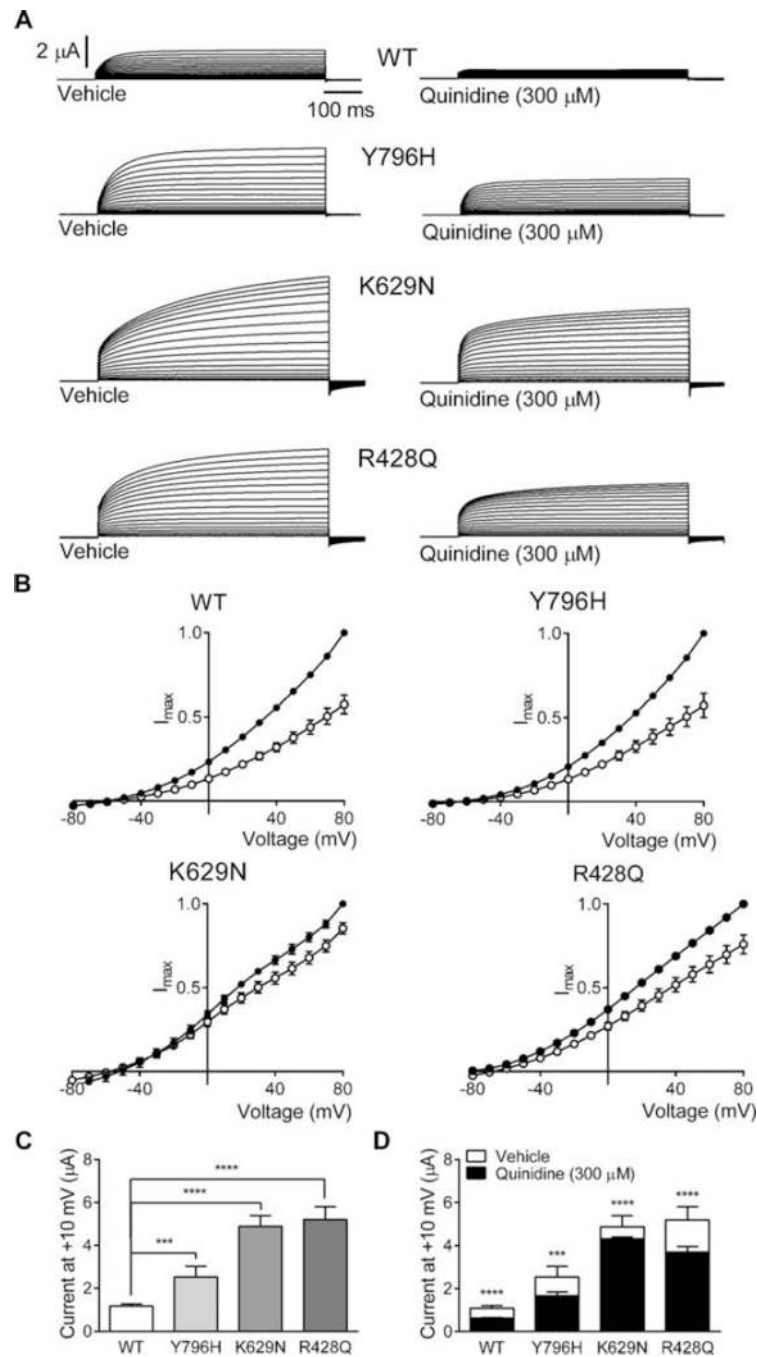
FIGURE 1.

Quinidine inhibition of h*KCNT1* currents expressed in *Xenopus* oocytes.[17,18] (A) Representative current traces obtained from oocytes expressing wild-type (WT), Y796H, K629N, and R428Q channels in vehicle control and in the presence of 300 μM quinidine. Scale bars apply to all traces. Oocytes were held at −90mV and stepped from −80mV to 80mV for 600 milliseconds every 5 seconds. (B) Normalized average current–voltage relationships for WT, Y796H, K629N, and R428Q channels in the presence of vehicle (*solid circles*) and 300 μM quinidine (*open circles*; WT, n = 20; Y796H, n = 9; K629N, n = 16;

R428Q, n = 23). Currents were normalized to the value in the absence of quinidine at a test potential of +80 mV (I_{max}). (C) Average peak currents at +10mV for WT (n = 127), Y796H (n = 63), K629N (n = 118), and R428Q (n = 38) channels. The peak currents for each mutant channel at +10mV were compared to the peak currents for the WT channel at +10mV. (D) Comparison of average current amplitude for WT and mutant channels illustrating the degree of block by 300 μM quinidine (WT, n = 13; Y796H, n = 9; K629N, n = 43; R428Q, n = 24) derived from measurements made at +10mV (see C for vehicle control n values; ***p< 0.001; ****p< 0.0001). Data for Y796H and R428Q are reformatted from our other study,[5] but are the same underlying data. Data are presented as mean-± standard error of the mean, and Student t test was used to test statistical significance.
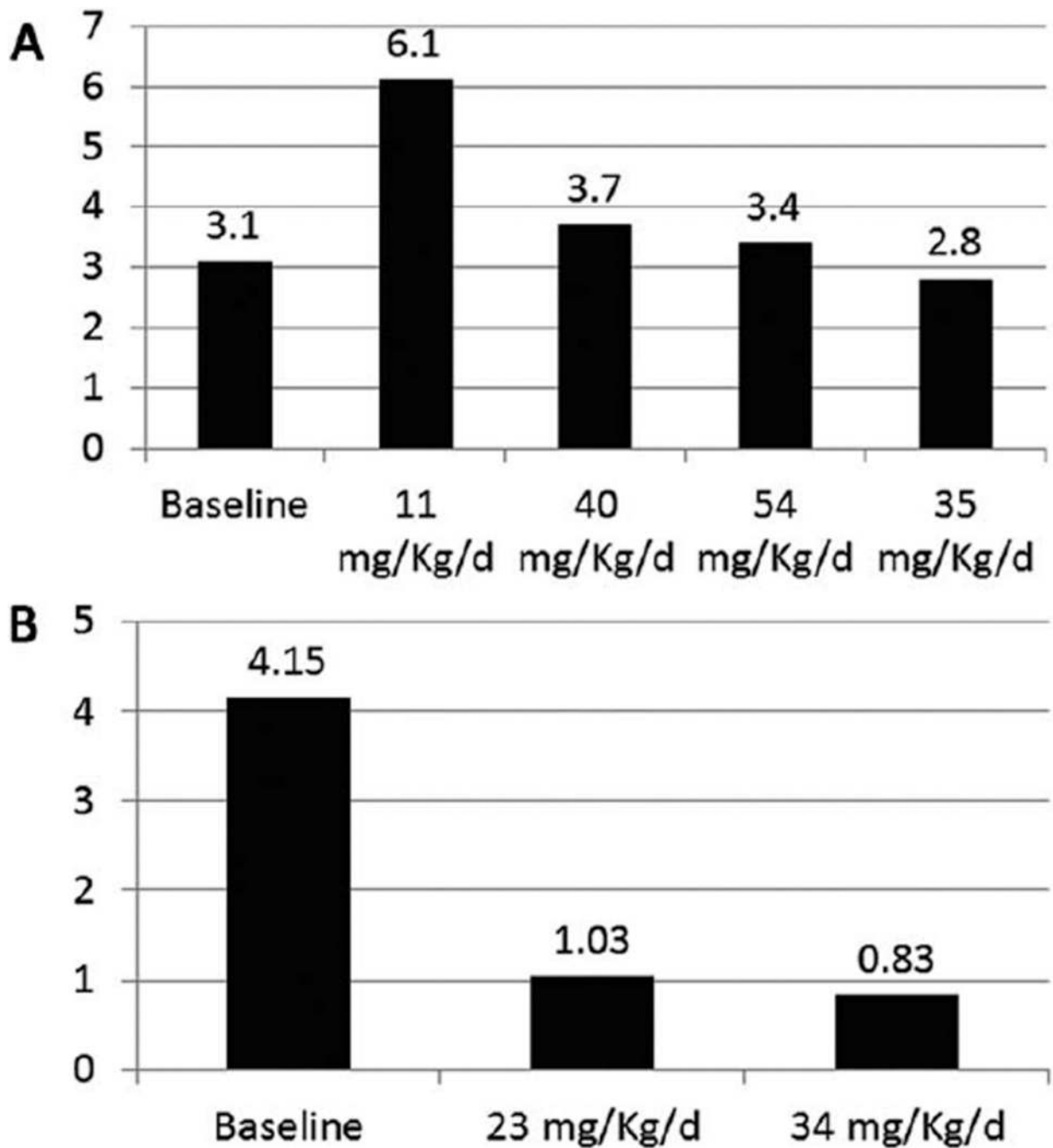
FIGURE 2.

Changes in seizure frequency during quinidine therapy in Patients 1 (A) and 2 (B). Seizure frequency was documented by review of the patient's daily seizure calendar, which we had established that the families were keeping reliably before the start of the baseline period. Baseline was the 1 month before starting quinidine therapy, and average baseline daily seizure frequency was calculated over that month. During quinidine therapy, seizure frequency was calculated over the period of the stable dose of quinidine, which was always for a minimum of 1 month or longer if the patient was kept on the specific stable quinidine

dose for a longer period of time. We monitored both of our patients with inpatient video-electoencephalographic recordings before initiation of the quinidine therapy to confirm the nature of the seizures and that the families were reliably recognizing seizures, and did not identify any striking discrepancies. For an individual patient, over the course of the follow-up, there was no difference in their seizure type, severity, or duration based on the families' reports and based on the inpatient monitoring that also occurred throughout the inpatient hospitalizations during dose increases.