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FULL-LENGTH ORIGINAL RESEARCH

Lack of response to quinidine in *KCNT1*-related neonatal epilepsy

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Summary

Objective: To evaluate the clinical efficacy and safety of quinidine in patients with *KCNT1*-related epilepsy of infancy with migrating focal seizures (EIMFS) in the infantile period and to compare with the effect of quinidine on mutant channels in vitro.

Methods: We identified 4 patients with EIMFS with onset in the neonatal period, pathogenic variants in the *KCNT1* gene, and lack of response to AEDs. Patients were prospectively enrolled, treated with quinidine, and monitored according to a predefined protocol. Electroclinical, neuroimaging, and genetic data were reviewed. Two patients had novel variants in the *KCNT1* gene that were modeled in *Xenopus* oocytes with channel properties characterized using electrophysiology recordings.

Results: Three of four patients were treated with quinidine early in their disease course, prior to 6 months of age. No significant side effects were noted with quinidine therapy. In addition, there were no significant changes in electroencephalography (EEG)–confirmed seizure burden during therapy, and patients had near hourly seizures before, during, and after treatment. Two patients had previously reported gain-of-function mutations, which demonstrated sensitivity to high levels of quinidine in the oocyte assay. Two patients with novel variants, showed characteristic gain-of-function and were thus predicted to be pathogenic. Of interest, these variants were essentially insensitive to high levels of quinidine.

Significance: Patients had no reported benefit to quinidine therapy despite age at treatment initiation. Pharmacogenetic results in oocytes were consistent with clinical treatment failure in 2 patients, suggesting that single-dose pharmacologic assessment may be helpful in predicting which patients are exceedingly unlikely to achieve benefit with quinidine. In the 2 patients who had a lack of therapeutic benefit despite sensitivity to high concentrations of quinidine with in vitro *oocyte assay*, it is likely that the achievable exposure levels in the brain were too low to cause significant in vivo channel blockade.

KEYWORDS
electrophysiology, epilepsy of infancy with migrating focal seizures, epileptic encephalopathy, *KCNT1*, precision medicine

Numis, Nair, Petrou, and Cilio contributed equally to the manuscript.

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1 | INTRODUCTION

Epilepsy of infancy with migrating focal seizures (EIMFS) is a severe early onset epileptic encephalopathy with high morbidity. Patients typically have seizure onset in the first 6 months of age, with a clinical course characterized by intractable epilepsy and severe intellectual disability.[1,2]

The most common cause are gain-of-function pathogenic variants in the *KCNT1* gene, encoding a sodium-activated potassium channel called Slack (sequence like a calcium-activated potassium channel).[3,4] Activation of Slack results in K^+ efflux, contributing to the modulation of action-potential firing behaviour.[5] Enhanced Slack current seen in EIMFS patients could trigger epileptogenic firing properties by shortening action-potential duration and firing rate.[6–8] Variants in the *KCNT1* gene are also associated with disparate epilepsy syndromes, including autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) as well as other focal epilepsies with variable phenotypes.[9,10] However, variants found in patients with EIMFS cause more severe perturbations in channel conductance than in ADNFLE, up to 5 times greater than normal.[11]

Seizures in EIMFS are multifocal, prolonged, and arise independently and sequentially from both hemispheres, occur many times each day, and are refractory to nearly all conventional antiepileptic drugs. Some seizure control has been reported with potassium bromide or levetiracetam.[12–14] The discovery that the class I antiarrhythmic drug quinidine can reverse channel overactivity in vitro led to its rapid clinical application in a patient with EIMFS and an R428Q (c1283G>A, pArg428Gln) variant with reported in vivo efficacy.[11,15] Subsequently, reductions in seizure frequency have been observed in few patients, some with functionally milder gain-of-function mutations in *KCNT1*, but treatment failures in other patients were also reported.[16–20] A dissociation with regard to in vitro efficacy of quinidine on potassium current and in vivo responsiveness has been noted, with a distinct patient with an R428Q variant demonstrating the least effective seizure reduction with quinidine.[17] These findings raise the question about determinants of responsiveness to quinidine, including phenotype, time from symptom onset to the initiation of treatment, or dose.

Here, we report 4 patients with *KCNT1*-associated EIMFS, all with onset in the neonatal period, including 2 patients with novel variants. All patients were treated with quinidine according to a standardized protocol, including 2 patients treated before 2 months of age. We report on clinical safety and efficacy for all patients and in vitro efficacy for the 2 previously unreported mutations.

Key Points

- EIMFS can present in the neonatal period and is associated with gain-of-function pathogenic variants in the *KCNT1* gene
- Quinidine may reverse the effects of pathogenic *KCNT1* variants in vitro
- Quinidine has no impact in seizure burden in patients, even when trialed early in the course of the disease
- Seizure frequency and severity improve with potassium bromide, but the long-term developmental prognosis remains unaltered

2 | METHODS

2.1 | Clinical characteristics

2.1.1 | Patient identification

Patients with the electroclinical phenotype of EIMFS and refractory to multiple antiepileptic drugs (AEDs) were identified at the participating centers.[1] As clinically indicated, patients underwent genetic testing with commercially available panels of common epilepsy-associated genes utilizing Sanger sequencing of genomic DNA.[21] Information was obtained directly from the referring physicians. The original clinical, ictal, and interictal video-electroencephalography (EEG), brain magnetic resonance imaging (MRI), and antiepileptic drug (AED) treatment of all patients were reviewed by pediatric neurologists and neurophysiologists (ALN, AD, MRC). Developmental delay and cognitive impairment were assessed using developmental milestones, current functioning, and neurologic examination. The degree of cognitive impairment was assessed qualitatively with a developmental quotient (DQ) based on acquisition of developmental milestones.

The study was approved by the human research ethics committee of the University of California, San Francisco. Parents assented for off-label use of quinidine for the treatment of epilepsy.

2.1.2 | Quinidine treatment

Infants with EIMFS associated with *KCNT1* variants (NM_020822.2) characterized by epileptic encephalopathy and intractable seizures were prospectively approached and trialed on quinidine using a standardized protocol. In brief, prior to quinidine initiation, patients were admitted to the cardiac step-down unit on continuous video-EEG and electrocardiography (ECG) telemetry, a standard 12-lead ECG was performed, and patients were given a test dose of

quinidine of 2 mg/kg. During admission and prior to quinidine initiation, patients were evaluated with 24-hour video-EEG recording for determination of seizure type, frequency, and duration. Baseline complete blood count (CBC), complete metabolic panels, concomitant AED trough levels, and β-hydroxybutyrate when the ketogenic diet was co-administered were evaluated at baseline. After no side effects were reported to the initial test dose, patients were started on quinidine 15 mg/kg/d divided into 4 doses (maximum 200 mg/dose). Doses were escalated by 15 mg/kg/d at 14-16 day intervals up to a dose of 100 mg/kg/d, as tolerated, with the goal serum quinidine level between 2 and 5 mg/L.

Prior to each dose increase, patients were admitted for inpatient video-EEG and ECG telemetry to reevaluate seizure burden. In 3 of 4 patients, blood was obtained during each admission for quinidine trough levels, concomitant AED trough levels, and β-hydroxybutyrate when the ketogenic diet was coadministered. A CBC and complete metabolic panel were obtained prior to and 24-48 hours after each dose escalation. Once on stable dosing of quinidine, patients were reevaluated with video-EEG and ECG telemetry every 3 months. All ECGs were reviewed by a board-certified pediatric cardiologist.

2.2 | Electrophysiology

2.2.1 | Oocyte preparation

Xenopus oocytes (Dumont stage V or VI) were surgically removed from *Xenopus laevis* and prepared as described previously.[22] Oocytes were kept in ND96 solution and stored at 17°C. Fifty nanoliters of capped complementary RNA (cRNA) was injected into each oocyte using the Roboocyte system (Multi Channel Systems, Reutlingen, Germany). A total of 10 ng of cRNA was injected into each oocyte.

2.2.2 | KCNT1 mutagenesis and in vitro transcription

A259D (c.776 C>A) and A259V (c.776 C>T) constructs were obtained from Genewiz in a Miniprep form. R1114W (c.3340 C>T) and Del550 (c.1649-1651delAGC, p. Gln550del) mutations were introduced into human *KCNT1* expression construct[11] using QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, California) according to the manufacturer's instructions. Construct fidelity for all 4 variants was confirmed by DNA sequencing. cDNAs were transcribed in vitro (mMessage mMachine; Ambion, Austin, Texas).

2.2.3 | Electrophysiology recordings

Two-electrode voltage-clamp recording was performed using the Roboocyte System (Multi Channel Systems) after 14-48 hours of expression. Electrodes containing 1.5 mol/L K-acetate and 0.5 mol/L KCl were used to impale oocytes that were held at −90 mV and perfused with bath solution containing (in millimolars) 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, and 5 HEPES, pH 7.5. Recording frequency was 1 kHz, and temperature was maintained between 20 and 22°C. To record expressed membrane currents, oocytes were held at −90 mV, and test depolarizations lasting for 1000 millisecond were applied at 10 mV increments, from −110 mV to +80 mV, at 5-second intervals. Contemporaneous measurement of wild-type (WT) *KCNT1* currents was performed with the same batch of oocytes injected with variant *KCNT1* messenger RNA (mRNA) to maintain an internal control of possible variability in expression from different batches of oocytes. Quinidine (Sigma, St. Louis, Missouri) was dissolved in ethanol to a concentration of 300 μmol/L and was perfused continuously to the oocytes for 1 minute, followed with 5-minute incubation. Currents were recorded before and after application.

The results obtained were compiled to depict functional differences of each variant (A259D, A259V, R1114W, and Q550del) and the effect of quinidine on A259D, R1114W, and Q550del in comparison to WT (and A259D vs A259V, Figure S1). Relative current traces, current voltage relationship curves and time to 50% peak curves were employed to determine functional differences between WT and mutants. To determine the effect of quinidine, in addition to relative current traces and current voltage relationship curves, the percent of remaining current for mutants (A259D, R1114W, and Q550del) and WT was evaluated, after exposure to the compound.

AxoGraph (AxoGraph Scientific, Sydney, Australia) was used to analyze the electrophysiologic data while statistical analysis was performed using Graphpad Prism 6 (GraphPad Software, La Jolla, California). Results are presented as a mean ± standard error of the mean. Statistical significance for current voltage relationship curves and time to 50% peak was determined using 2-way analysis of variance (ANOVA) with Bonferroni post hoc analysis, whereas Student *t* test was used to determine significant differences in percent of remaining current between WT and A259D mutant. One-way ANOVA with Bonferroni post hoc analysis was used to determine significant differences in percent of remaining current between WT, R1114W, and Q550del mutants.

3 | RESULTS

3.1 | Case descriptions

All patients had seizure onset in the neonatal period within 2 weeks of birth. No patient had pre- or perinatal risk factors for seizures, including evidence of hypoxic-ischemic

encephalopathy. Seizures were initially focal and rapidly evolved into prolonged multifocal seizures. EEG was initially characterized by multifocal interictal epileptiform discharges, evolving into subcontinuous ictal discharges migrating from one region to another and from one brain hemisphere to another. Seizure duration was approximately 1-3 minutes and patients had multiple seizure per day. Most patient had clusters of multiple seizures per hours. Patients had failed several AEDs at the time of initiation of quinidine (Table 1). Patients began treatment between 1.5 months and 30 months of age, with 3 of 4 patients starting treatment within 5 months of age. At the time of treatment, all patients had nearly hourly seizures, often in clusters, lasting 30 seconds to 2 minutes, intractable to multiple AEDs.

Extensive metabolic and neuroradiologic testing were unrevealing. Genetic testing showed predicted disease-causing variants in *KCNT1* in all patients. Mutations were reported previously and characterized in 2 patients (R428Q [c1283G>A][16] and M516V [c1546 A>G][23]). One patient had a novel variant in *KCNT1* at A259D. One patient had one novel variant in *KCNT1*, a de novo in-frame Del550 and a paternally inherited recurrent population variant, R1114W.[24]

3.2 | Quinidine therapy

There were no significant changes in seizure frequency and severity during therapy, with patients having >1-2 seizures per hour before, during, and after quinidine therapy. Quinidine therapy was stopped before reaching 100 mg/kg/d or a level of 2-5 mg/L in 3 of 4 cases due to perceived inefficacy (Figure 1A,B). There were no significant side effects noted during quinidine therapy. There were modest increases in QTc noted with prolonged therapy, although none reached criteria for prolonged QT (Figure 1C). Quinidine therapy increased trough levels of potassium bromide in one patient and decreased levels in another patient, in a dose-independent fashion. Patients on quinidine therapy receiving concomitant phenobarbital, carbamazepine, potassium bromide, or ketogenic diet therapy had no sustained alterations in AED trough levels/β-hydroxybutyrate (Table S1). One patient had a 32%-37% decreases in levetiracetam levels over the course of quinidine therapy.

3.3 | Clinical follow-up

Patients have been followed for 12-24 months after quinidine initiation. At follow-up, one patient died at 15 months of age due to cardiac arrest. This patient had not received quinidine for >6 months and cause of death was presumed unrelated to quinidine. All other patients continued to have intractable epilepsy. Notably, in all patients, potassium bromide in add-on was associated with a decrease in seizure frequency in doses ranging from 50-80 mg/kg/d. All patients were diagnosed with profound developmental delay in keeping with the natural history of EIMFS.[25] Therefore, there were no significant changes in expected developmental trajectory, positive or negative, as a result of quinidine therapy.

3.4 | Functional characterization of *KCNT1* variants

To investigate the functional impact of the unreported *KCNT1* mutants (R1114W-Patient 1, Del550-Patient 1, and A259D-Patient 2) on channel properties, electrophysiologic analyses were performed in *Xenopus* oocytes. In Patient 1, the paternally inherited R1114W allele is a recurrent population variant and was functionally similar to the WT variant and should thus be considered benign. However, the de novo Del550 variant found in Patient 1 showed a surprising gain-of-function phenotype in both current magnitude and activation kinetics (Figure 2D-F) and is the likely causative mutation. In Patient 2, a de novo A259D variant was found, and functional assessment showed a characteristic gain-of-function in current magnitude and activation kinetics (Figure 2A-C) as seen in various other EIMFS patients. Previous reports have demonstrated *KCNT1* mutants expressing M516V (Patient 3) or R428Q (Patient 4) and result in gain-of-effect with increased K+ conductance compared to WT channels.[16,23]

3.5 | Quinidine sensitivity of *KCNT1* variants

To investigate the sensitivity of *KCNT1* variants to quinidine, the response to 300 μmol/L quinidine was measured in the oocyte assay. As reported previously, application of 300 μmol/L quinidine resulted in a significant reduction in current amplitudes of WT channels. In contrast, application of 300 μmol/L quinidine to the A259D and Q550del variant channels revealed a marked insensitivity. Previous reports have demonstrated that application of quinidine to cells expressing mutant M516V (Patient 3) or R428Q (Patient 4) resulted in more pronounced reductions in current amplitudes.[16,23]

4 | DISCUSSION

With improvements in cost and availability of genetic testing, there is an increase in the reporting of specific causative mutations in patients with epilepsy.[26] Here, we report on 2 novel variants of *KCNT1*, A259D and Q550del, with descriptions of both the clinical phenotype and pathologic channel properties. Although our advancements in

TABLE 1 Genetic, clinical, EEG characterization, and treatment response to quinidine

	Case 1	Case 2	Case 3	Case 4
Genetics				
KCNT1 mutation	(1) De novo c.1649-1651delAGC: p.Gln550del (Del550) (2) Paternal c.3340 C>T: p.Arg1114Trp (R1114W)	De novo, c.776 C>A: pAla259Asp (A259D)	De novo c1546 A>G: pMet516Val (M516V)	De novo c1283 G>A: pArg428Gln (R428Q)
Clinical characteristics				
GA at birth	38 3/7	39 5/7	41 0/7	Estimated 36, mother did not know of pregnancy until time of delivery
Seizure onset (day of life)	14	1	1	5
Maximum seizure frequency	>1-2/h	>3-4/h	>1-4/h	~1/h
Seizure types	Asymmetric focal tonic seizures ± clonic with tachycardia, up to 13 min; myoclonic; epileptic spasms.	Asymmetric tonic posturing associated with eye/head deviation with alternating sides	Asymmetric tonic posturing associated with eye/head deviation with alternating sides; tongue-thrusting in first month of life.	Type 1: Clonic movements of arms and legs with unilateral eye twitching (R or L),± lip smacking; Type 2: Eye deviation to right or left, with flushing and increased resp rate; Type 3: electrographic only
Other clinical	VSD, diffuse hypotonia	Diffuse hypotonia	Diffuse hypotonia	Diffuse hypotonia, right gaze preference
EEG characteristics				
Interictal background	Lack of organization, diffuse background slowing, abundant multifocal independent SW, PSW, SSW, and random synchronous attenuations.	Lack of organization, diffuse background slowing, abundant multifocal independent SW, random synchronous attenuations.	Lack of organization, diffuse background slowing, abundant multifocal independent SW and PSW	Lack of organization and no well-formed sleep architecture. Diffuse background slowing (delta/theta), frequent multifocal spikes SW
Ictal	Tonic: Cessation of interictal SW, background attenuation with multifocal onset and then emergence of rhythmic sharpened alpha with evolution to delta ±SW.	Tonic: Cessation of interictal SW, background attenuation with superimposed faster frequencies, multifocal in onset, then emergence of rhythmic sharpened alpha.	Tonic: Cessation of interictal SW, background attenuation with superimposed fastest frequencies, multifocal in onset, then emergence of focal rhythmic sharpened theta evolving into frank SW.	With tonic component (Type 2), there is cessation of interictal SW, background attenuation with superimposed faster frequencies, multifocal in onset, followed by emergence of Sharply contoured rhythmic theta with evolution to delta ±SW. (Type 1 and 3).

(Continues)

TABLE 1 (Continued)

	Case 1	Case 2	Case 3	Case 4
MRI findings	22 mo: Diffuse WM loss (progressive), diffuse T2-WM hyperintensities, MRS mild elevation in choline peak	5 mo: Severe diffuse CC thinning, mild diffuse low WM volume. MRS wnl.	1 mo: Diffuse low WM volume. MRS wnl.	3 mo: Normal
AEDs trialed (* denotes benefit)	CLB, KD, LEV, PB, PHT, TPM, B6, pharmaceutical-grade CBD, KBr (*80 mg/kg/d).	CLB, CZP, KGD, LEV, TPM, PB, PHT, KD, B6, folinic acid, pharmaceutical-grade CBD, KBr (*80 mg/kg/d).	CBZ (*), CLB, PB, TPM, B6, KBr (*50 mg/kg/d).	CBZ, CLB, LEV, OXC, TPM, PB (*), B6, KBr (*80 mg/kg/d).
Quinidine trial				
Age at initiation	5 mo	30 mo	2 mo	1.5 mo
Dose	Up to 100 mg/kg/d (max serum level 1.0)—Ineffective	Up to 80 mg/kg/d (max serum level 2.0)—ineffective	Up to 60 mg/kg/d (max serum level 4.8 mg/L)—ineffective	Up to 50 mg/kg/d (max level 1.3)—ineffective

Clinical characteristics: GA, gestational age. EEG: MF, multifocal; PSW, polyspike-wave; SW, spike-wave; SSW, slow spike-wave. MRI: CC, corpus callosum; MRS, MR spectroscopy; WM, white matter; Wnl, within normal limits. Medications: AEDs, antiepileptic drugs; B6, pyridoxine; CBZ, carbamazepine; CLB, clobazam; CZP, clonazepam; KBr, potassium bromide; KD, ketogenic diet; LEV, levetiracetam; OXC, oxcarbazepine; PB, phenobarbital; PHT, phenytoin; TPM, topiramate.

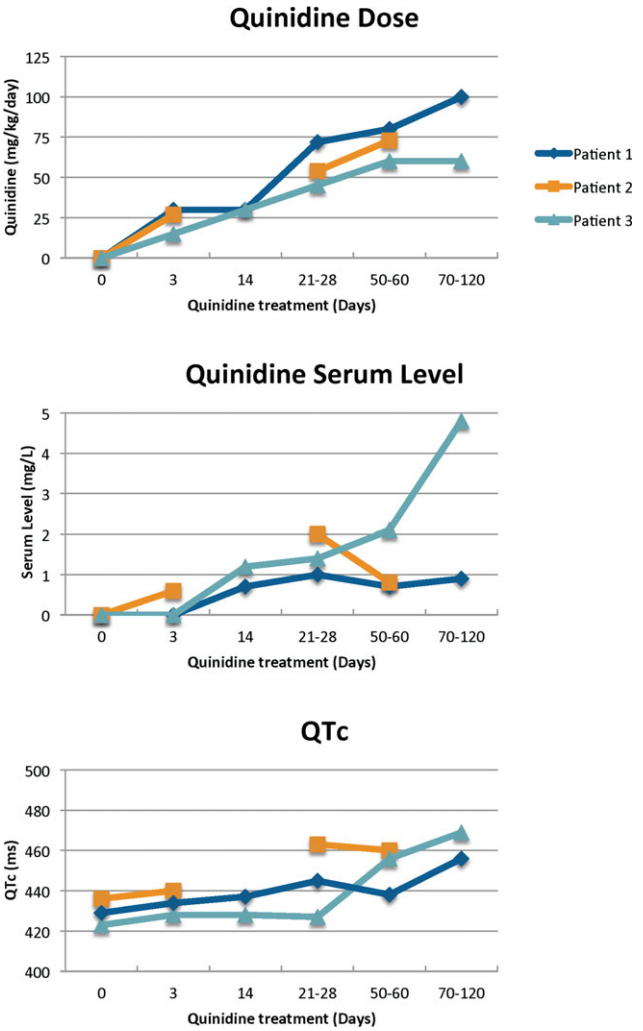

FIGURE 1 Association of quinidine dose, serum level, and QTc interval

diagnosis have improved our ability to convey prognosis, the model of precision medicine as it pertains to disease-specific treatments in early onset epileptic encephalopathies remains challenging. In *KCNQ2* and *SCN2A* encephalopathy, the use of sodium channel blockers can result in seizure freedom without substantial concomitant alterations in long-term neurodevelopmental prognosis, even when therapies are initiated within the first months of life.[27–29] In *KCNT1*-associated EIMFS, quinidine's action can effectively block the pathologic constitutive activation of the *KCNT1* channel at the molecular level in some variants.[11] Clinical efficacy has been inconsistent.[15–17] Similar results have been noted with *KCNT1*-related autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE).[30]

In this case series of 4 patients with neonatal-onset EIMFS associated with *KCNT1* pathogenic variants, quinidine therapy was safe, although it did not result in a reduction in clinical seizure frequency or duration and did not affect neurodevelopment. This is consistent with our in vitro

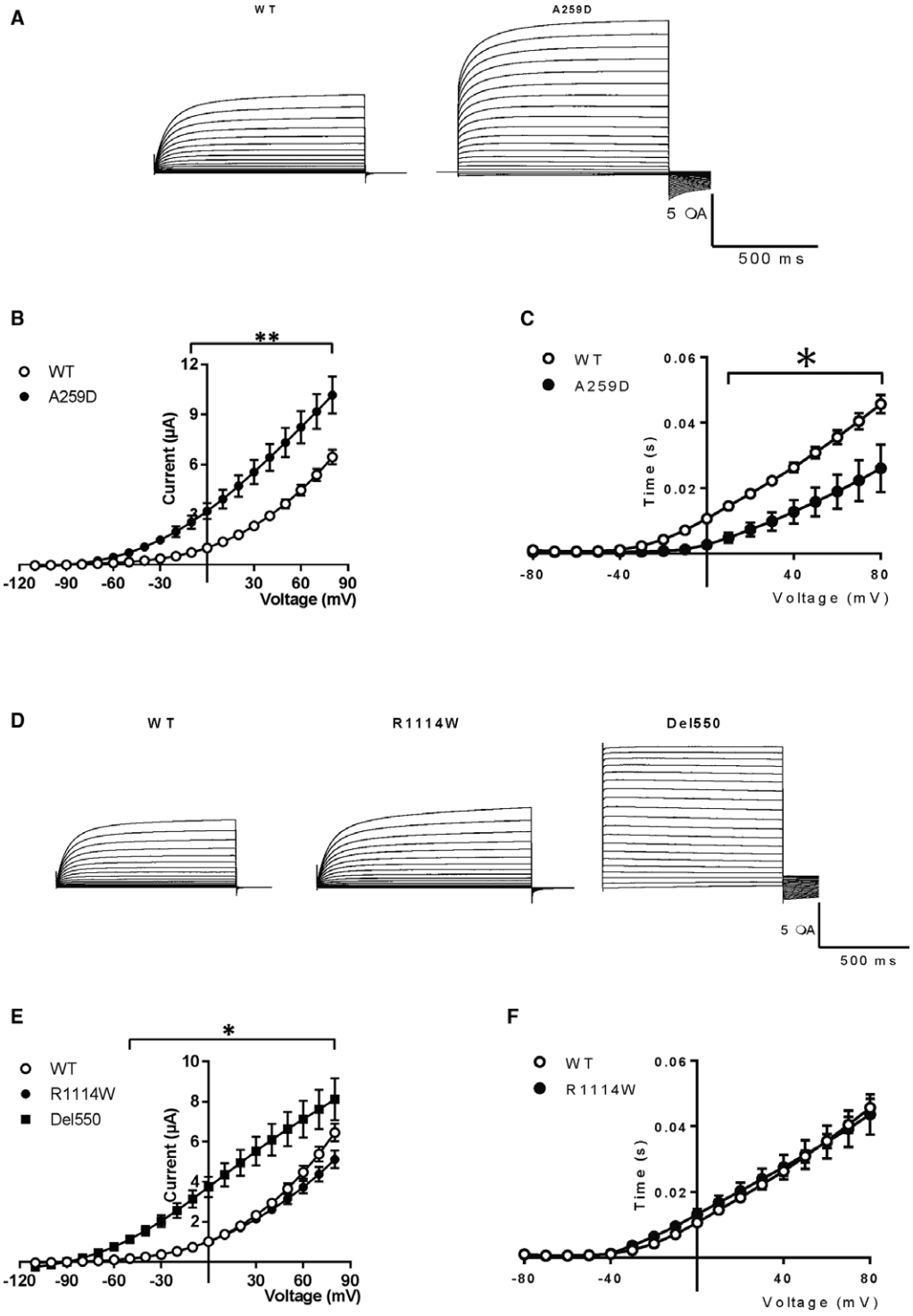

FIGURE 2 Human *KCNT1* WT, A259D (A-C), and R1114W/Del550 mutant (D-F) currents recorded in *Xenopus* oocytes. A, Representative current traces obtained from *Xenopus* oocytes expressing WT and A259D mutant and D, WT, R1114W, and Del550 mutant. Oocytes were held at −90 mV and stepped from −110 mV to +80 mV for 1000 msec every 5 s. Scale bars apply to all traces. B, Comparison of raw current–voltage relationships of WT (n = 37) and A259D (n = 25), revealed significantly larger currents for the mutant (2-way ANOVA followed by Bonferroni post hoc analysis). C, Time to 50% peak of currents for WT (n = 28) and A259D (n = 13) exhibited significantly lower time for A259D mutant compared to WT channels (2-way ANOVA followed by Bonferroni post hoc analysis). $*P < 0.1$, $**P < 0.01$. E, Comparison of raw current-voltage relationships of WT (n = 37), R1114W (n = 25), and Del550 (n = 27), revealed significantly larger currents for the deletion mutant (2-way ANOVA followed by Bonferroni post hoc analysis). F, Time to 50% peak of currents for WT (n = 28) and R1114W (n = 11) exhibited no difference between both channels (2-way ANOVA followed by Bonferroni post hoc analysis). Time to 50% peak for Del550 mutant was not accurate due to quick activation of channel. $*P < 0.1$

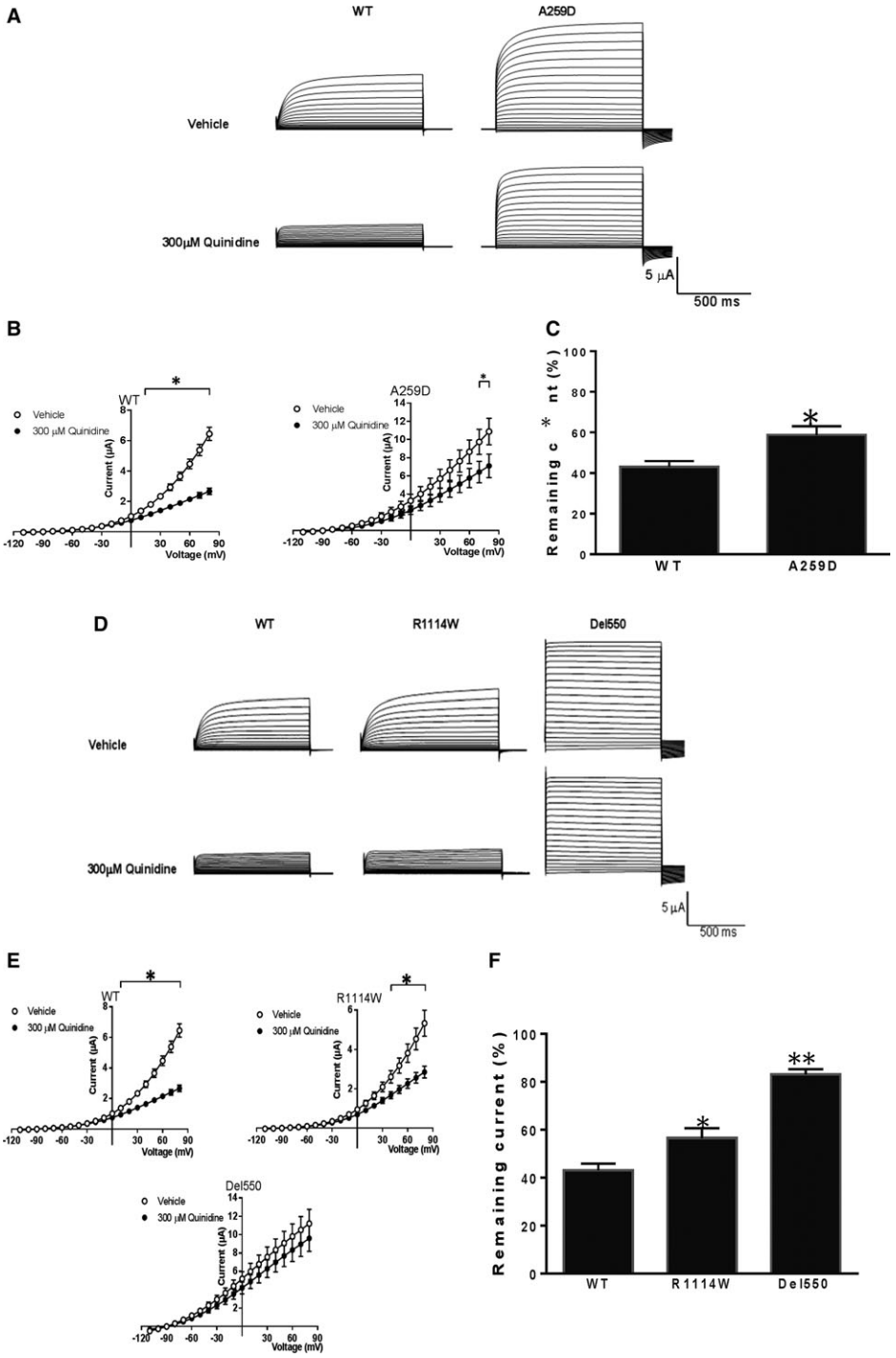

FIGURE 3 Application of quinidine onto *Xenopus* oocytes expressing human *KCNT1* WT, A259D (A-C), and R1114W/Del 550 mutants (D-F). A, Representative current traces obtained from *Xenopus laevis* oocytes expressing WT and A259D mutant and D, WT, R1114W, and Del550 mutant, each with application of vehicle (ND 96) and 300 μmol/L quinidine. Oocytes were held at −90 mV and stepped from −110 mV to +80 mV for 1000 ms every 5 s. Scale bars apply to all traces. B, Raw current-voltage relationships for WT (n = 37) and A259D (n = 15) before and after application of 300 μmol/L quinidine. Current amplitudes reduced significantly in WT and A259D channels (2-way ANOVA followed by Bonferroni post hoc analysis). C, Average remaining current at +80 mV of WT (n = 37) and A259D (n = 15) human *KCNT1* channels after application of 300 μmol/L quinidine showed significant difference between WT and A259D channels (unpaired *t* test). $*P < 0.1$, $**P < 0.01$. E, Raw current-voltage relationships for WT (n = 37), R1114W (n = 16) and Del550 (n = 14) before and after application of 300 μmol/L quinidine. Current amplitudes reduced significantly in WT and R1114 channels with exposure to quinidine (2-way ANOVA followed by Bonferroni post hoc analysis). F, Average remaining current at +80 mV of WT (n = 37), R1114W (n = 16), and Del550 (n = 14) h*KCNT1* channels after application of 300 μmol/L quinidine revealed significant differences between WT, R1114W, and Del550 (1-way ANOVA followed by Bonferroni post hoc analysis). $*P < 0.1$, $**P < 0.01$

results of variants from Patients 1 and 2 that harbored Q550del and A259D, which were essentially insensitive to even high doses of quinidine. This is an important finding that has implications for future drug discovery, as disease variants could have a range of sensitivity to novel or other repurposed medications. Patients harboring drug-insensitive mutations are unlikely to achieve much therapeutic benefit, and such assays should be incorporated in future precision medicine applications in *KCNT1*-positive EIMFS.

Patients 3 and 4 harbored mutations that displayed in vitro sensitivity to 300 μmol/L quinidine yet failed to derive any therapeutic benefit. This apparent mismatch in the in vitro and in vivo response of quinidine therapy may be explained by target-specific tissue concentrations. Quinidine penetrates across the blood–brain barrier, with levels varying between 9% and 46% of unbound plasma levels.[31,32] After a therapeutic dose of quinidine is administered orally, cerebrospinal fluid concentrations range from 9-15 nmol/L. In modeled human *KCNT1* mutants, 300 μmol/L is necessary to produce a significant block.[11] The clinical inefficacy we observed may then be due to inadequate tissue concentrations. We targeted serum quinidine levels to established therapeutic levels of 2-6 μg/mL. Toxicity can arise at any dose, but certainly at serum levels above 9 μg/mL. Therefore, further increases in serum levels are unlikely to result in adequate target tissue concentrations without significant cardiovascular side effects. Coupled with our poor appreciation of the extent of *KCNT1* blockade needed for clinical benefit, it is not surprising that quinidine was ineffective. A better understanding of exposure and necessary receptor occupancy may tailor future in vitro assays to assess the efficacy of novel therapeutics at lower concentration ranges and to predict clinical efficacy in patients with *KCNT1* variants.

Prior case series have described patients with seizure onset in the neonatal period in *KCNT1*-associated EIMFS, although only one case has been diagnosed so early on in the disease course.[18] All patients in this series initiated quinidine therapy at an early age, with 2 patients initiating therapy within the first 2 months of life.[15–18,33] Despite early targeting of the pathologic channel, no disease-modifying effects were noted in the clinical phenotype. In contrast to previous hypotheses, our data do not support "age at quinidine initiation" as a variable that can modify overall outcome.[19] Perhaps then, single gene mutations cause more widespread problems that targeted therapy even when initiated at an early age in development cannot overcome.

Although targeting of the *KCNT1* channel with quinidine in EIMFS did not provide benefit, genomic-guided therapy with potassium bromide may ameliorate the epileptic phenotype in this population. Consistent with prior series, all patients here who received potassium bromide were noted to have reductions in seizure frequency and severity.[12,13] Although as in *KCNQ2* encephalopathy and *SCN2A* encephalopathy, we do not anticipate bromides to ultimately affect long-term neurodevelopmental prognosis.

The precise mechanism of hyperexcitability driven by mutations in the *KCNT1* gene remains unknown. Does quinidine therapy then truly target the deleterious effects resulting in the epileptic phenotype? The *KCNT1* protein contains a long C-terminal domain allowing for interactions with numerous cytosolic proteins including the protein kinase C (PKC) and fragile-x mental retardation (FRMP) proteins, which independently increase the likelihood of channel opening.[34,35] We are in need of novel therapeutic strategies that either more specifically target disease genes or impact the network scale changes seen in the early onset epileptic encephalopathies. Early and effective diagnosis, with detailed electroclinical phenotyping and genetic testing, still yields the best chances of targeted therapy in a window where we can truly exert changes to neurodevelopment. However, this relationship is undoubtedly complex.

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DISCLOSURES OF CONFLICTS OF INTEREST

Dr. Oldham is currently affiliated with Medpace, Cincinnati, OH. Dr Petrou is a cofounder and equity holder of Praxis Precision Medicines (Cambridge, Massachusetts) and RogCon (Miami Beach, Florida). He is on the Scientific Advisory Board member and equity holder of Pairnomix (Minneapolis, Minnesota). Dr. Cilio receives research funding, paid to her Institution, from Insys Therapeutics for company-sponsored trials, and received fees and travel support for serving in a scientific advisory board from BioMarin Pharmaceuticals. The remaining authors have no conflicts of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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