

RESEARCH ARTICLE

Screening of conventional anticonvulsants in a genetic mouse model of epilepsy

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Abstract

Objective: Epilepsy is a common neurological disorder that affects 1% of the population. Approximately, 30% of individuals with epilepsy are refractory to treatment, highlighting the need for novel therapies. Conventional anticonvulsant screening relies predominantly on induced seizure models. However, these models may not be etiologically relevant for genetic epilepsies. Mutations in *SCN1A* are a common cause of Dravet Syndrome, a severe epileptic encephalopathy. Dravet syndrome typically begins in infancy with seizures provoked by fever and then progresses to include afebrile pleomorphic seizure types. Affected children respond poorly to available anticonvulsants. *Scn1a*^{+/-} heterozygous knockout mice recapitulate features of Dravet syndrome and provide a potential screening platform to investigate novel therapeutics. In this study, we conducted a screening of conventional anticonvulsants in *Scn1a*^{+/-} mice to establish assays that most closely correlate with human response data. **Methods:** On the basis of clinical response data from a large, single center, retrospective survey of Dravet syndrome case records, we selected nine drugs for screening in *Scn1a*^{+/-} mice to determine which phenotypic measures correlate best with human therapeutic response. We evaluated several screening paradigms and incorporated pharmacokinetic monitoring to establish drug exposure levels. **Results:** *Scn1a*^{+/-} mice exhibited responses to anticonvulsant treatment similar to those observed clinically. Sodium channel blockers were not effective or exacerbated seizures in *Scn1a*^{+/-} mice. Overall, clobazam was the most effective anticonvulsant in *Scn1a*^{+/-} mice, consistent with its effect in Dravet syndrome. **Interpretation:** Genetic models of spontaneous epilepsy provide alternative screening platforms and may augment the AED development process. In this study, we established an effective screening platform that pharmacologically validated *Scn1a*^{+/-} mice for preclinical screening of potential Dravet syndrome therapeutics.

Introduction

Epilepsy is a common neurological disorder with a lifetime prevalence of 2–5%. While some individuals with epilepsy achieve adequate seizure control with currently available anticonvulsant drugs, approximately, 30% remain refractory to treatment, highlighting the need for novel drugs.¹ Anticonvulsant development has relied predominantly on screening for activity against specific seizure types in acute seizure models, mainly maximal

electroshock and pentylenetetrazole (PTZ).² Acute seizure models are limited by not being etiologically relevant to the approximately two-thirds of epilepsies categorized as genetic in origin. Genetic models of epilepsy exhibit spontaneous seizures, like humans, and their usage may increase the potential of therapy screening processes for drug-resistant epilepsies.

Dravet syndrome is a severe epileptic encephalopathy, most often resulting from *de novo* *SCN1A* mutations.³ Seizure onset typically begins in the first year of life,

often provoked by fever. Individuals subsequently develop multiple seizure types that are refractory to treatment. Dravet syndrome also includes developmental delays, cognitive impairment, motor dysfunction, and an increased risk for sudden unexpected death in epilepsy (SUDEP).^{3,4} Typical pharmacotherapies include benzodiazepines, valproic acid, and stiripentol, but response is usually suboptimal.^{5–7} Lamotrigine and carbamazepine, both sodium channel inhibitors, are not recommended for Dravet syndrome as they have been reported to exacerbate seizures.^{8,9}

The *Scn1a*^{+/-} heterozygous null mouse model recapitulates many features of Dravet syndrome, including spontaneous seizures, seizures provoked by hyperthermia, and premature mortality.^{10–12} To characterize this model as a potential platform to evaluate therapeutic compounds, we conducted a screen of conventional anticonvulsants to determine which phenotypic measure(s) correlated best with human response data ascertained from a retrospective survey of clinical records from the Epilepsy Center at Ann & Robert H. Lurie Children's Hospital of Chicago. Nine conventional anticonvulsants were evaluated in *Scn1a*^{+/-} mice for effects on hyperthermia-induced seizures, spontaneous generalized tonic-clonic seizures (GTCS), and survival. Pharmacokinetic monitoring was used to evaluate the effect of drug doses that produced plasma concentrations within the human therapeutic range. We determined that lamotrigine worsened, while clobazam, valproic acid, stiripentol, levetiracetam, and phenobarbital improved the seizure phenotype of *Scn1a*^{+/-} mice. This is mainly consistent with what is observed clinically in Dravet syndrome. Our results demonstrate feasibility of using a genetic epilepsy model to screen for anticonvulsant responses and validate a phenotyping platform for evaluating novel therapeutics using the *Scn1a*^{+/-} mouse model of Dravet syndrome.

Materials and Methods

Chart review

Approval for this study was obtained from the Ann & Robert H. Lurie Children's Hospital Internal Review Board (IRB) prior to data collection. We performed a retrospective chart review of all files where "SCN1A" or "Dravet" was documented in the diagnosis or problem list since inception of electronic medical records (EMR) at Children's Hospital in 2007. Inclusion criteria included a genetic or clinical diagnosis of Dravet syndrome, a suspected pathogenic variant in *SCN1A*, and epilepsy consult visit at Lurie Children's Hospital since 2007. Patients with a GEFS+ diagnosis or an *SCN1A*-negative diagnosis of Dravet syndrome were excluded.

Treatment response data were coded based on patient/parent-reported seizure frequency outcome.

Animals

All animal care and experimental procedures were approved by the Northwestern University Animal Care and Use Committee in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The principles outlined in the ARRIVE (Animal Research: Reporting of in vivo Experiments) guideline and Basel declaration (including the 3R concept) were considered when planning experiments. Mice were group-housed in a specific pathogen-free mouse facility under standard laboratory conditions (14-h light/10-h dark) and had access to food and water ad libitum, except during hyperthermia-induced seizure experiments. *Scn1a*^{+/-} mice were generated as previously described and were maintained as a congenic line on the 129S6/SvEvTac (129.*Scn1a*^{+/-}) background.¹¹ For experiments, F1 mice were produced by breeding heterozygous 129.*Scn1a*^{+/-} mice with wild-type C57BL/6J mice (Jackson Laboratory stock 000664). The *Scn1a* genotype was determined by as previously described.¹¹

Drugs

Carbamazepine, clobazam, lamotrigine, levetiracetam, phenobarbital, topiramate, and valproic acid were purchased from Sigma-Aldrich Co. (St. Louis, MO). Stiripentol (Sigma-Aldrich Co.; Cayman Chemical, Ann Arbor, MI) and phenytoin sodium solution (X-Gen Pharmaceuticals, Horseheads, NV) were also used. For acute administration, drugs were prepared as solutions with the following vehicles: A- saline (levetiracetam, phenobarbital, topiramate, valproic acid); B- 0.5% methylcellulose in water (phenytoin); C- 5% hydroxypropyl- β -cyclodextrin (carbamazepine, lamotrigine); and D- vegetable oil (clobazam, stiripentol). All drugs were administered as a single intraperitoneal (ip) injection in a volume of 10 mL/kg. The following acute doses resulted in plasma concentrations within the human therapeutic range (Table 1): 20 mg/kg carbamazepine, 5 mg/kg clobazam, 20 mg/kg lamotrigine, 10 mg/kg levetiracetam, 15 mg/kg phenobarbital, 15 mg/kg phenytoin, 40 mg/kg topiramate, 25 mg/kg stiripentol, 75 mg/kg valproic acid, and 0.1–10 mg/kg clobazam plus 25 mg/kg stiripentol. Acute toxicity was not observed with any drugs at the administered doses.

Analytical methods

Carbamazepine, clobazam, lamotrigine, levetiracetam, phenobarbital, and stiripentol in plasma samples were assayed by high performance liquid chromatography

Table 1. Plasma concentrations and doses of anticonvulsants.

	Human therapeutic range plasma concentration (total)	Hyperthermia assay plasma concentration (dose, mg/kg body weight)	Subchronic treatment plasma concentration (dose, mg/kg of chow)
Carbamazepine	4–12 $\mu\text{g/mL}$	$8.7 \pm 0.7 \mu\text{g/mL}$ (20 mg/kg)	$5.3 \pm 0.9 \mu\text{g/mL}$ (7500 mg/kg chow)
Clobazam	0.1–0.4 $\mu\text{g/mL}$ (NCLB: 0.3–3 $\mu\text{g/mL}$) ¹	$0.4 \pm 0.1 \mu\text{g/mL}$ (NCLB: $1.3 \pm 0.1 \mu\text{g/mL}$) (5 mg/kg)	BQL (NCLB: $0.6 \pm 0.03 \mu\text{g/mL}$) (160 mg/kg chow)
Lamotrigine	3–15 $\mu\text{g/mL}$ ²	$8.5 \pm 0.4 \mu\text{g/mL}$ (20 mg/kg)	$3.5 \pm 0.7 \mu\text{g/mL}$ (125 mg/kg chow)
Levetiracetam	10–37 $\mu\text{g/mL}$ ¹	$35.3 \pm 2.8 \mu\text{g/mL}$ (10 mg/kg)	$33.6 \pm 5.7 \mu\text{g/mL}$ (1350 mg/kg chow)
Phenobarbital	15–40 $\mu\text{g/mL}$ ¹	$18.8 \pm 2.4 \mu\text{g/mL}$ (15 mg/kg)	$18.4 \pm 2.7 \mu\text{g/mL}$ (300 mg/kg chow)
Phenytoin	10–20 $\mu\text{g/mL}$ ¹	$12.0 \pm 0.6 \mu\text{g/mL}$ (15 mg/kg)	N/a
Stiripentol	8–16 $\mu\text{g/mL}$ ^{3,4}	$13.1 \pm 3.7 \mu\text{g/mL}$ (25 mg/kg)	$12.3 \pm 2.6 \mu\text{g/mL}$ (4000 mg/kg chow)
Topiramate	5–25 $\mu\text{g/mL}$ ¹	$23.6 \pm 1.2 \mu\text{g/mL}$ (40 mg/kg)	$13.3 \pm 0.9 \mu\text{g/mL}$ (850 mg/kg chow)
Valproic Acid	50–150 $\mu\text{g/mL}$ ⁵	$122 \pm 5 \mu\text{g/mL}$ (75 mg/kg)	$71 \pm 4 \mu\text{g/mL}$ (4500 mg/kg chow)
Clobazam + Stiripentol	CLB: 0.1–0.4 $\mu\text{g/mL}$ (NCLB: 0.3–3 $\mu\text{g/mL}$) STP: 8–16 $\mu\text{g/mL}$	BQL (NCLB: BQL) (CLB 0.1–1 mg/kg + STP 25 mg/kg)	$10.20 \pm 3.6 \mu\text{g/mL}$ (NCLB: $1.1 \pm 0.4 \mu\text{g/mL}$) (CLB+STP: 160 mg + 4000 mg per kg chow)

Dosage listed in parenthesis. Data expressed as mean \pm SEM, with $n = 5$ –8 samples per group.

BQL, Below limit of quantification; NCLB, *N*-desmethylclobazam.

¹Clin Chem Lab Med 2004,42(11):1228-55.

²Ther Drug Monit 2003, 25(3):347-63.

³Clin Pharmacokinet 2015, 54(5):527-36.

⁴Lancet 2000, 356(9242):1638-42.

⁵Goodman & Gilman's The Pharmacological Basis of Therapeutics 2006.

(HPLC) based on previously described methods with modifications.^{13–18} Details regarding sample preparation and chromatographic conditions for each drug are provided in Table S1 and Figure S2. Briefly, plasma samples (50–100 μL) were spiked with internal standard (prazepam, caffeine or hexobarbital) and extraction was achieved by vortex-mixing with 4 \times volume of organic solvent. The organic layer was isolated by centrifugation at 376g for 5 min and was evaporated to dryness by heating at 75°C. The residues were reconstituted in mobile phase for analysis with an HPLC 9 Flexar Binary LC Pump Platform and Flexar UV/Vis Detector (Perkin Elmer, Waltham, MA). Quantitative analysis was performed by comparing samples to standards prepared with known amounts of drug.

Plasma phenytoin concentration was assayed by fluorescence polarization immunoassay (COBAS INTEGRA; Roche, Basel, Switzerland) by Vanderbilt University Medical Center Core Chemistry Laboratory.

Plasma topiramate and valproic acid concentrations were determined using ARK Topiramate Assay (ARK Diagnostics, Inc., Fremont, CA) and *Emit* 2000 Valproic Acid Assay (Beckman Coulter, Inc., Brea, CA), respectively. Each sample (1 μL) was mixed with 58 μL of Reagent 1 and 29 μL of Reagent 2 and incubated for 15 min at room temperature. Reactions were terminated with 1 mmol/L DHEA (dehydroepiandrosterone), a potent inhibitor of glucose-6-phosphate dehydrogenase, and placed on ice.¹⁹ Absorbance

was measured spectrophotometrically at 340 nm (Nano-Drop 8000; Thermo Fisher Scientific, Inc., Waltham, MA). Quantitative analysis was performed by comparing samples to standards prepared with known amounts of drug.

Pharmacokinetic analysis

We performed pharmacokinetic studies of clobazam and stiripentol to establish doses in *Scn1a*^{+/-} mice that resulted in plasma drug concentrations within the human therapeutic range. Wild-type and *Scn1a*^{+/-} mice received a single ip injection of 10 mg/kg clobazam or 100 mg/kg stiripentol. At selected time points (5–120 min for clobazam; 0–300 min for stiripentol), mice were anesthetized with isoflurane and whole blood was collected by cardiac puncture. Plasma was isolated by centrifugation (9000g for 10 min, 4°C) and stored at –80°C until assayed. Plasma drug concentrations at each time point were averaged and pharmacokinetic parameters were calculated by noncompartmental analysis. The linear-log trapezoidal method was used to estimate total drug exposure (area under concentration-time curve) using the following equation:

$$\text{AUC} = \text{Absorption AUC} + \text{Elimination AUC} + \text{Terminal AUC}$$

$$\text{Absorption AUC} = \sum_{t_1 \rightarrow t_{\text{max}}} \frac{(C_1 + C_2) \cdot (t_2 - t_1)}{2}$$

$$\text{Elimination AUC} = \sum_{t_{\max} \rightarrow t_{\text{last}}} \frac{(C1 - C2)}{([\ln(C1) - \ln(C2)])} \cdot (t2 - t1)$$

$$\text{Terminal AUC} = \frac{C_{\text{last}}}{k_e}$$

Elimination rate constant (k_e) was determined by linear regression of the terminal component of the concentration-time curve using GraphPad Prism 6.07 (La Jolla, CA).

The above pharmacokinetic parameters were used for calculating dosages and determining target experimental time points for the hyperthermia-induced seizure protocol and for subchronic oral treatment dosing during spontaneous seizure studies. Pharmacokinetic parameters of stiripentol in plasma were determined as follows: C_{\max} (46 $\mu\text{g}/\text{mL}$), t_{\max} (90 min), $t_{1/2}$ (169 min), and $\text{AUC}_{0 \rightarrow \infty}$ (12625 $\mu\text{g} \cdot \text{min}/\text{mL}$) (Fig. S1A). Clobazam pharmacokinetic parameters were determined as follows: C_{\max} (2.4 $\mu\text{g}/\text{mL}$), t_{\max} (25 min), $t_{1/2}$ (38 min), and $\text{AUC}_{0 \rightarrow \infty}$ (155 $\mu\text{g} \cdot \text{min}/\text{mL}$) (Fig. S1B). Clobazam-treated mice were also assayed for *N*-desmethylclobazam, an active metabolite of clobazam. Following treatment with clobazam, the C_{\max} and t_{\max} of *N*-desmethylclobazam were 3.1 $\mu\text{g}/\text{mL}$ and 90 min, respectively. Exposure of both clobazam and *N*-desmethylclobazam were considered when determining experimental schemes for clobazam treatment.

Hyperthermia-induced seizures

Hyperthermia-induced seizure experiments were conducted in *Scn1a*^{+/-} mice at age postnatal day 14-16 (P14-16) using a rodent temperature regulator (TCAT-2DF, Physitemp Instruments, Inc, Clifton, NJ) reconfigured with a Partlow 1160 + controller (West Control Solutions, Brighton, UK) connected to a heat lamp and RET-3 rectal temperature probe. Fifteen minutes prior to the target experimental (post-dose) time point for each drug, the rectal probe was inserted. Mice acclimated to the temperature probe for 5 min before the hyperthermia protocol was started. Mouse core body temperature was elevated 0.5°C every 2 min until the onset of the first clonic convulsion with loss of posture or until 42.5°C was reached. Mice that reached 42.5°C were held at temperature for 3 min. If no seizure occurred during the hold period, the mouse was considered seizure-free. After thermal induction procedure, plasma samples were isolated as described above and stored at -80°C until assayed. Threshold temperatures were compared using the time to event analysis (logrank Mantel-Cox), and $P < 0.05$ was considered statistically significant. No significant sex differences were observed, so groups were collapsed across sex.

Experimental time points were based on previously determined time-to-peak plasma and brain concentrations or effect from the literature or our pharmacokinetic studies. Experimental time points used were as follows: 40 min-levetiracetam; 45 min- carbamazepine, lamotrigine, phenobarbital; 90 min- stiripentol, clobazam, topiramate; 120 min phenytoin.²⁰⁻²⁴ Valproic acid was administered after the 5 min acclimation period.^{25,26} Matched vehicle controls were run for each experimental time point. Vehicle A (saline) was administered at 0, 40, 45, and 90 min. No statistical difference was identified between the four time points and all vehicle A-treated mice were combined into one group. Vehicles B (0.5% methylcellulose), C (5% hydroxypropyl- β -cyclodextrin), and D (vegetable oil) were administered at 120, 45, and 90 min, respectively.

Spontaneous seizure frequency monitoring

Frequency of spontaneous generalized tonic-clonic seizures (GTCS) was quantified in untreated and drug-treated *Scn1a*^{+/-} mice. Anticonvulsant compounds were administered orally through supplementation in chow. Lamotrigine (125 mg per kg of chow) was formulated in Purina 5LOD by Research Diets (New Brunswick, NJ). Carbamazepine (7500 mg per kg of chow), clobazam (160 mg per kg of chow), levetiracetam (1350 mg per kg of chow), phenobarbital (300 mg per kg of chow), stiripentol (4000 mg per kg of chow), topiramate (850 mg per kg of chow), valproic acid (4500 mg per kg of chow), and clobazam plus stiripentol (160 mg and 4000 mg per kg of chow, respectively) were formulated in-house in Purina 5LOD irradiated meal (Lab Diets, St. Louis, MO). Chow formulations for conventional anticonvulsants resulted in plasma concentrations within the human therapeutic range for each drug (Table 1). Acute toxicity was not observed with any drug at the administered doses.

At P18, *Scn1a*^{+/-} mice were randomly assigned to drug or control treatment groups with ad libitum access to drug-containing or control chow. At P20, mice were housed in groups of three in a recording chamber (28 × 28 × 36 cm) and overhead video was captured using a Day/Night camera (Samsung SCB5003) equipped with an infrared lens (Tamron 13FG04IRSQ). During recording, mice had ad libitum access to food and water, and were maintained on a 14:10 light-dark cycle. Spontaneous GTCS were captured by continuous video recording over 60 h (12:00 on P22 through 24:00 on P24). Digital video images captured during the 60-h video-monitored session were analyzed offline by an observer blinded to treatment. Previous video-EEG monitoring of *Scn1a*^{+/-} mice verified a perfect correlation between behavioral and electroencephalographic seizures ($\kappa = 1.0$), therefore we relied on video monitoring for our primary screening.²⁷ Mice continued

drug treatment and survival was monitored until P30. Plasma was then isolated between 6:00 and 8:00am and drug levels were assayed as described above. Statistical comparisons were made using Fisher's exact test (proportion seizure-free), Kruskal–Wallis with Dunn's post hoc test (seizure frequency), and logrank (Mantel-Cox) test (survival) with GraphPad Prism and $P < 0.05$ was considered statistically significant. Mice that died between P18 and P19 were excluded to allow for adequate drug exposure time. No significant sex differences were observed, so groups were collapsed across sex.

Spontaneous GTCS following hyperthermic seizure priming

Prior to evaluating spontaneous GTCS frequency, untreated P18 *Scn1a*^{+/-} mice were exposed to a single hyperthermia-induced seizure as described above. At the onset of clonic convulsion with loss of posture, mice were immediately placed on a cooling pad to terminate the seizure. The average temperature at seizure onset was 42.2 ± 0.05 (range: 41.1–43.1). Mice were cooled to a core body temperature of 37°C and then randomly assigned to drug treatment groups (untreated, clobazam, stiripentol, valproic acid, or clobazam plus stiripentol) administered through supplementation in chow, as described above. Due to the higher subsequent seizure burden and earlier onset in primed *Scn1a*^{+/-} mice, we shifted the monitoring window for spontaneous seizures to begin at an earlier time point. Spontaneous GTCS were captured by continuous video recording from 12:00 on P19 through 24:00 on P21. The number of spontaneous GTCS captured during the 60-h video-monitored session was quantified offline by an observer blinded to treatment. Mice continued drug treatment and survival was monitored until P30 at which time plasma was isolated as described above. Statistical comparisons were made using Fisher's exact test (proportion seizure-free) and logrank (Mantel-Cox) test (survival analysis) with GraphPad Prism and $P < 0.05$ was considered statistically significant. No significant sex differences were observed, so groups were collapsed across sex. Among drug treatment groups, there was no difference in the temperature at which the initial hyperthermia-induced seizure occurred ($F(3,59) = 2.046$, $P = 0.5804$, one-way ANOVA). Additionally, we performed a pilot experiment to verify that following a thermally induced seizure, mice consume the expected amount of drug-containing chow and achieve sufficient drug exposure by P19. We exposed a small cohort of *Scn1a*^{+/-} mice to a hyperthermia-induced seizure on P18 and then commenced treatment with phenobarbital chow. Plasma drug levels were measured 24 h post

seizure induction and compared to naïve littermate mice treated with phenobarbital chow in parallel by student's t-test. There was no difference ($P < 0.480$) between plasma levels in the primed and naïve mice, validating that feeding behavior is not significantly disrupted by the hyperthermia-priming procedure (primed: 17.8 ± 3.2 µg/mL; naïve: 23.4 ± 5.6 µg/mL).

Results

Anticonvulsant responses in Dravet syndrome

We performed a retrospective analysis of anticonvulsant drug responses among a cohort of Dravet syndrome cases seen in the Epilepsy Center at Ann & Robert H. Lurie Children's Hospital of Chicago between 2007 and 2016. An initial search of the EMR with the terms "SCN1A" or "Dravet" identified 211 records, which were then surveyed for a confirmed clinical diagnosis of Dravet syndrome and a pathogenic/likely pathogenic *SCN1A* variant. This resulted in a total of 133 distinct medical records that were included in the study. The mean age at the most recent visit was 8.4 years (standard deviation (SD) 5.6; range 1–26), and the mean age of first recognized seizure was 5 months (SD 2; range <1–12). The mean number of current AEDs per patient was 3 (SD 1) and the mean number of AEDs tried was 7 (SD 3). Response data based on patient or parent-reported outcome was coded for each AED trial, irrespective of other medications administered concurrently, and the percentage in each response category was tabulated for each drug that was trialed in a minimum of 30 patients. The relative efficacy of each medication was calculated as the percentage of children taking the medication whose parents reported reduction in seizure frequency. In our Dravet syndrome cohort, we found >50% of patients had an effective response with tolerable side effects during treatment with clobazam (63/90), valproic acid (66/113), or stiripentol (26/38), while less than 45% had effective response with tolerable side effects with clonazepam (14/33) or topiramate (36/98) (Fig. 1). Zonisamide (46 total), levetiracetam (107 total), and phenobarbital (83 total) had limited effectiveness. Sodium channel blockers produced significant worsening of seizures in approximately 50% of patients trialed on these drugs, including lamotrigine (25/50), phenytoin (15/31), carbamazepine (16/32), and oxcarbazepine (28/50) (Fig. 1). This analysis is consistent with what has been reported in the literature from other epilepsy centers.^{5–7,28,29}

Based on this retrospective analysis, we compiled a list of representative drugs to test anticonvulsant response in the *Scn1a*^{+/-} mouse model using a variety of assays, including hyperthermia-induced seizures, spontaneous seizure monitoring, and survival.

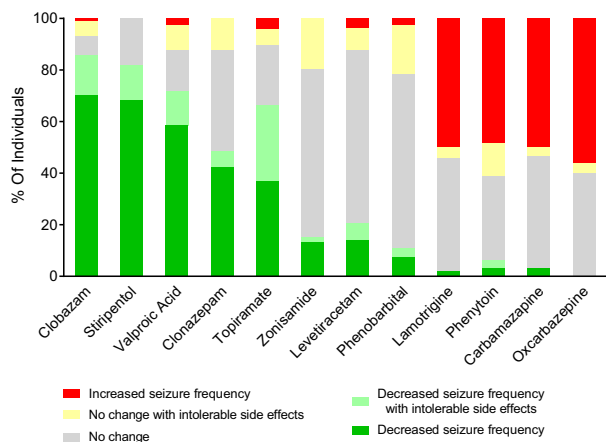


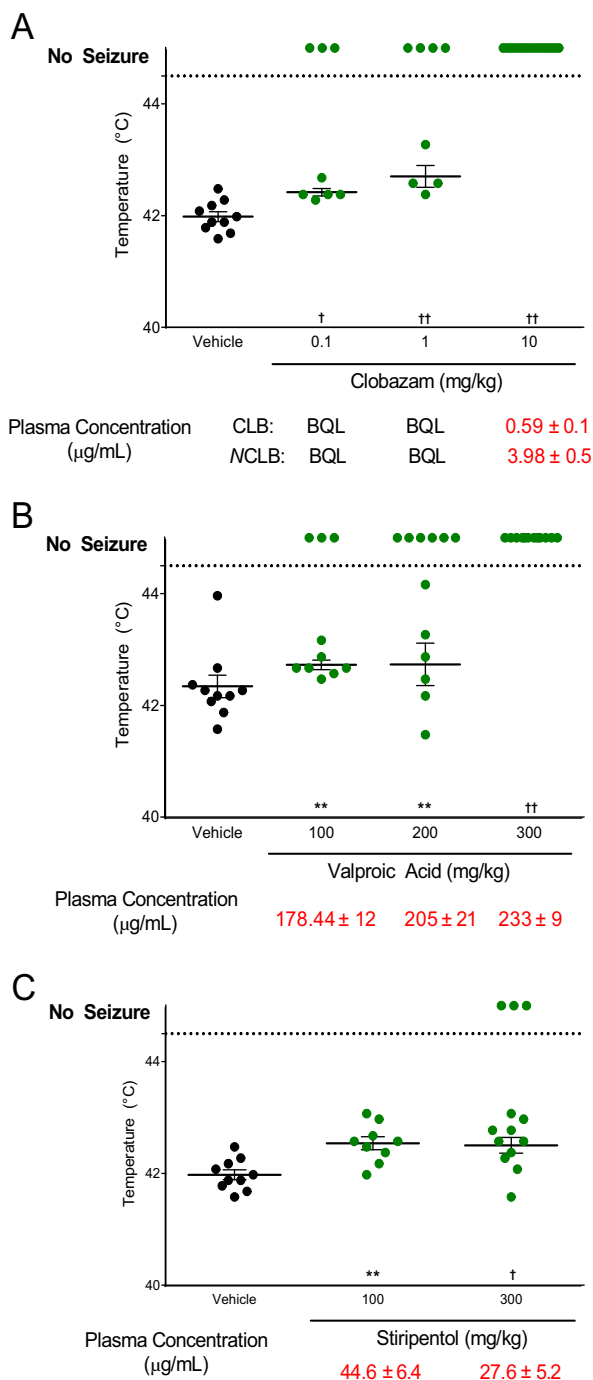
Figure 1. Seizure frequency response to anticonvulsants in a Dravet syndrome clinical population. Parent-reported changes in seizure frequency in pediatric patients carrying a diagnosis of clinical Dravet syndrome were quantified over the course of anticonvulsant therapy treatment and normalized per the size of the patient population treated. Red bars indicate an overall increase in seizure frequency. Yellow bars denote a lack of change with medication, limited by duration of therapy or dose limitations due to side effects considered intolerable by parents. Dark and light green indicate decreased number of seizures without side effects, or with duration of therapy or dose limitations due to side effects, respectively. Gray bars indicate the proportion of patients with no change in seizure frequency.

Evaluation of anticonvulsant drugs against hyperthermia-induced seizures

Children with Dravet syndrome and *Scn1a*^{+/-} mice both exhibit seizures in response to elevated body temperature. To determine if mouse and human anticonvulsant responses were correlated, we evaluated the dose-effect of valproic acid, clobazam, and stiripentol, which have been shown to provide some degree of therapeutic benefit in Dravet syndrome. Between P14 and P16, *Scn1a*^{+/-} mice were treated acutely by IP injection of a single compound or vehicle and

Figure 2. Dose-effect screening using hyperthermia-induced seizures in *Scn1a*^{+/-} mice. Threshold temperature of individual mice for GTCS induced by hyperthermia following acute treatment with varying doses of drug. Average plasma concentrations ± standard error of the mean (SEM) are listed beneath the corresponding drug dose. (A) Clobazam significantly protected against hyperthermia-induced GTCS at doses within and beyond the human therapeutic range. (B) Valproic acid provided significant protection against hyperthermia-induced GTCS at doses that result in plasma concentrations above the human therapeutic range. (C) Stiripentol provided significant protection against hyperthermia-induced GTCS at doses resulting in plasma concentrations above the human therapeutic range. The average temperatures of seizure induction are depicted by the thick horizontal line. Error bars represent SEM, with *n* = 8–17 per group (***P* < 0.01, †*P* < 0.001, ††*P* < 0.0001, logrank Mantel-Cox). [N.B.- Panels A and C use the same vehicle controls that are replotted for clarity.]

then subjected to hyperthermia. Plasma levels were measured after completion of the assay. For all three drugs, we observed dose-dependent protection against hyperthermia-induced seizures in *Scn1a*^{+/-} mice. Significant protection from hyperthermia-induced GTCS was achieved with clobazam plasma exposure levels within the human therapeutic range, with 37.5% protection at 0.1 mg/kg and 50% protection at 1 mg/kg (*P* < 0.0005, *P* < 0.0001, respectively)



(Fig. 2A). Complete protection was achieved at 10 mg/kg which resulted in plasma concentrations above the human therapeutic range ($P < 0.0001$). Valproic acid also provided significant dose-dependent protection, with 30% protection at 100 mg/kg ($P < 0.004$), 50% protection at 200 mg/kg ($P < 0.002$), and 100% protection at 300 mg/kg ($P < 0.0001$) (Fig. 2B). All three doses resulted in plasma concentrations above the human therapeutic range. Stiripentol provided significant protection compared to vehicle-treated controls, with an increased threshold temperature at 100 mg/kg ($P < 0.001$) and 23% (3/13) protected at 300 mg/kg ($P < 0.0001$), although these doses produced supratherapeutic plasma concentrations (Fig. 2C).

We also performed additional single-point analysis of a larger panel of conventional anticonvulsant drugs to determine effect on hyperthermia-induced seizure thresholds in *Scn1a*^{+/-} mice (Fig. 3). Acute single-point doses (IP administration) were selected to achieve plasma concentrations within the human therapeutic range and measured plasma levels are reported in Table 1. Consistent with the dose-effect results above, clobazam at 5 mg/kg provided significant protection, with 92% (11/12) seizure-free compared to 11% (2/18) of vehicle-treated controls (vehicle D, $P < 0.0001$). Significant protection from hyperthermia-induced GTCS was also achieved with levetiracetam and phenobarbital, with 50% (6/12) and 100% (12/12) seizure-free, respectively, compared to 16% (3/19) seizure-free vehicle-treated controls (vehicle A, $P < 0.019$ levetiracetam; $P < 0.0001$ phenobarbital). Treatment with lamotrigine, an anticonvulsant contraindicated in Dravet syndrome, significantly worsened the hyperthermia-induced seizure response. All 12 mice experienced a GTCS compared to 10 of 12 vehicle-treated controls, with a lower threshold temperature for lamotrigine-treated mice ($40.5 \pm 0.3^\circ\text{C}$) compared to vehicle-treated mice ($41.8 \pm 0.1^\circ\text{C}$) (vehicle C; $P < 0.0004$). Topiramate, phenytoin, carbamazepine, stiripentol, and valproic acid had no effect at the administered doses targeting the human therapeutic range.

Spontaneous seizure frequency in *Scn1a*^{+/-} mice

Scn1a^{+/-} mice exhibit spontaneous GTCS beginning at approximately age P16 with highest frequency between P21 and P25. We quantified spontaneous GTCS frequency in *Scn1a*^{+/-} mice subchronically treated with anticonvulsant drugs in chow. Drug treatment in *Scn1a*^{+/-} mice began at P18 and spontaneous GTCS were counted over 60 h of continuous video recording commencing 4 days later (12:00 on P22 through 24:00 on P24). Similar to the effect observed on

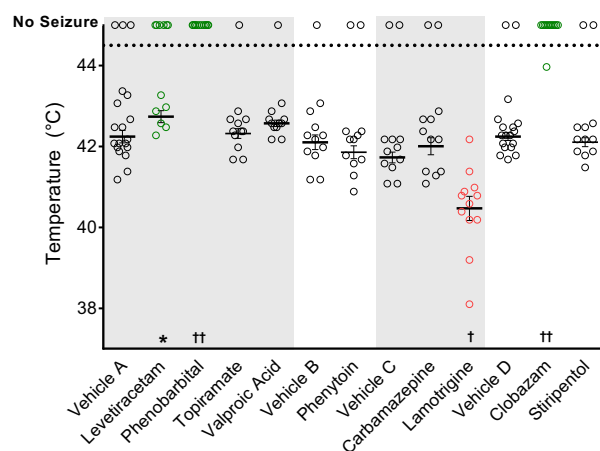


Figure 3. Single-dose anticonvulsant screening using hyperthermia-induced seizures in *Scn1a*^{+/-} mice. Threshold temperature of individual mice for GTCS induced by hyperthermia following acute administration of anticonvulsant doses to achieve plasma concentrations within the human therapeutic range. Lamotrigine treatment significantly lowered the threshold for thermally induced seizures and significantly increased the percentage of mice with GTCS (red, open symbols). Levetiracetam, phenobarbital, and clobazam treatments protected against GTCS, resulting in a significantly improved response to thermal seizure induction (green, open symbols). Vehicles A, B, C, and D correspond to saline, methylcellulose, HP β CD, and vegetable oil, respectively. The average temperatures of seizure induction are depicted by the thick horizontal line. Error bars represent SEM, with $n = 12$ – 19 per group (* $P < 0.05$, $^{\dagger}P < 0.001$, $^{\dagger\dagger}P < 0.0001$, logrank Mantel-Cox).

hyperthermia-induced seizures, lamotrigine treatment significantly worsened GTCS frequency ($P < 0.004$) (Fig. 4). Subchronic treatment with lamotrigine resulted in an average seizure rate of 6.9 ± 1.6 seizures per day, compared to controls, which had 2.0 ± 1.0 seizures per day. Lamotrigine was also associated with a higher proportion of mice experiencing GTCS during the observation period ($P < 0.014$).

Although subchronic treatment with clobazam, levetiracetam, phenobarbital, or valproic acid did not result in a statistically significant change in GTCS frequency, there was a trend toward protection against seizures. The lack of a significant response may be due to a floor effect, as the untreated *Scn1a*^{+/-} mice had relatively low seizure incidence and frequencies. This may be influenced, in part, by ascertainment bias, as a considerable number of untreated mice died prior to the P22 recording window. Regardless of the reason, this observation method was underpowered to identify compounds that significantly reduced spontaneous GTCS frequency within the observation window. Therefore, we developed a novel paradigm to improve our power to more efficiently discriminate a protective effect on spontaneous GTCS.

Spontaneous GTCS frequency in *Scn1a*^{+/-} mice following hyperthermic seizure priming

Dravet syndrome often begins with seizures provoked by fever and progresses to other seizure phenotypes, including afebrile GTCS. We modeled this seizure progression using a hyperthermia-induced seizure as a clinically relevant trigger to prime *Scn1a*^{+/-} mice. At P18, a single, brief GTCS was induced with the hyperthermia induction protocol described above. After GTCS onset, *Scn1a*^{+/-} mice were rapidly cooled back to 37°C, then drug treatment was initiated with anticonvulsant drugs in chow. Spontaneous GTCS were counted over 60 h of continuous video recording (12:00 on P19 through 24:00 on P21). This refined hyperthermia-priming protocol significantly improved our power to efficiently identify compounds that protect against spontaneous GTCS and more closely modeled the clinical situation where treatment is initiated after patients present with seizures. Comparing the naïve spontaneous seizure monitoring to the new paradigm of hyperthermia-priming in untreated mice revealed a significant increase in the proportion of *Scn1a*^{+/-} mice with subsequent unprovoked seizures, with 61% (11/18) of primed mice exhibiting seizures versus 24% (4/17) of naïve mice (Fig. 5) ($P < 0.041$).

Valproic acid, clobazam, and stiripentol consistently provide modest therapeutic benefit in Dravet syndrome, as observed both in our clinical data and the literature.^{6,7,30,31} Based on the available clinical information, we prioritized these drugs for further screening in our refined hyperthermia-priming paradigm. Following induction of a single, brief thermal seizure, we counted

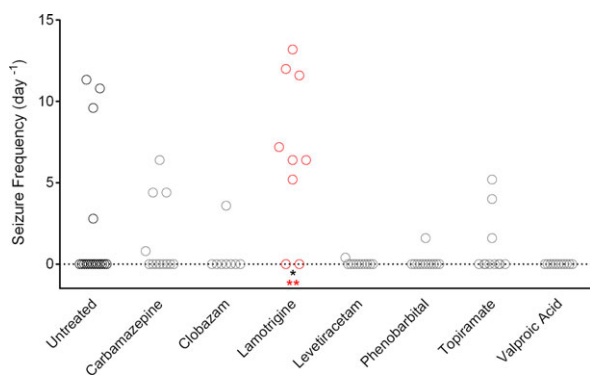


Figure 4. Screening anticonvulsant response using spontaneous seizure monitoring in *Scn1a*^{+/-} mice. GTCS frequency of individual untreated and drug-treated mice determined for a 60-hour period ($n = 8$ –17 mice per treatment group). Lamotrigine treatment resulted in a significantly higher seizure frequency and percentage of mice with seizures compared to untreated controls (** $P < 0.004$ (red), Dunn's multiple comparisons test; * $P < 0.014$, Fisher's exact test).

subsequent unprovoked GTCS in *Scn1a*^{+/-} mice subchronically treated with valproic acid, clobazam, or stiripentol using doses that targeted the human therapeutic range. Only subchronic treatment with clobazam offered a protective benefit against spontaneous seizures, with only 25% (4/16) of clobazam-treated *Scn1a*^{+/-} mice exhibiting spontaneous GTCS compared to 61% (11/18) of untreated controls ($P < 0.046$) (Fig. 5). It is perhaps unsurprising that subchronic treatment with valproic acid or stiripentol did not provide protective benefit with plasma concentrations in the human therapeutic range as our hyperthermia assay suggested that supratherapeutic levels of these drugs are required for significant seizure protection.

Anticonvulsant treatment alters GTCS severity

While monitoring spontaneous GTCS frequency in both the naïve and hyperthermia-primed *Scn1a*^{+/-} mice, we noticed distinct effects on seizure presentation and severity with lamotrigine, carbamazepine, topiramate, and valproic acid treatment. Therefore, in addition to quantifying seizure frequency, we analyzed whether subchronic anticonvulsant treatment modified seizure severity. We scored each spontaneous GTCS for advancement to the most severe stage of full tonic hindlimb extension (hindlimbs at a 180° angle to the torso). In the first cohort of naïve *Scn1a*^{+/-} mice that underwent seizure frequency analysis, three anticonvulsants significantly

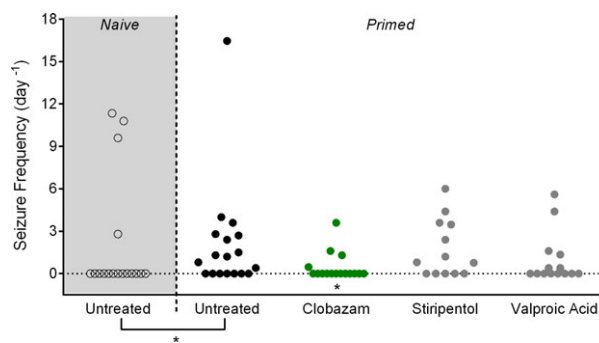


Figure 5. Screening anticonvulsant response using spontaneous seizure monitoring following hyperthermia-priming in *Scn1a*^{+/-} mice. GTCS frequency of individual untreated and drug-treated mice ($n = 13$ –18 mice per treatment group). Drug treatment was initiated following the induction of a single hyperthermia-induced seizure. Unprovoked, spontaneous GTCS were subsequently quantified over a 60-hour recording period. Clobazam treatment significantly reduced the proportion of mice experiencing GTCS (* $P < 0.046$, Fisher's exact test). The untreated cohort represented by black, open symbols, and gray shading are naïve controls to demonstrate the significant difference between naïve and hyperthermia-priming (black, closed circles) seizure incidences ($P < 0.041$, Fisher's exact test).

reduced the proportion of seizures that advanced to tonic hindlimb extension (Fig. 6A). Interestingly, while lamotrigine treatment significantly exacerbated GTCS frequency, the severity of the seizures was significantly reduced ($P < 0.0001$). None of the 155 seizures occurring during lamotrigine treatment escalated to tonic hindlimb extension, compared to 33% (17/52) of seizures in untreated controls. Seizures in lamotrigine-treated mice did not advance to hindlimb extension, but rather ended with vigorous clonic movements of fore- and hindlimbs while mice lay on their sides spinning in circles (Videos S1, S2). Carbamazepine treatment also significantly reduced the proportion of seizures that ended in tonic hindlimb extension ($P < 0.0001$). Carbamazepine-treated mice experienced several myoclonic jerks before progressing to GTCS with loss of posture, but no tonic hindlimb extension (Video S3). Additionally, treatment with topiramate significantly reduced the proportion of seizures that advanced to tonic hindlimb extension ($P < 0.004$). Seizures in topiramate-treated mice included several instances of rearing and paddling without loss of posture or tonic hindlimb extension (Video S4). In the

hyperthermia-primed cohort of *Scn1a*^{+/-} mice that underwent seizure monitoring, valproic acid treatment significantly reduced the proportion of seizures that advanced to tonic hindlimb extension ($P < 0.046$) (Fig. 6B). However, no other changes in seizure semiology were observed in valproic acid-treated mice. Seizure semiology and severity in all other drug-treated cohorts were not different from untreated *Scn1a*^{+/-} mice.

Survival analysis

Scn1a^{+/-} mice have a significantly reduced lifespan with the greatest proportion of mice dying between the third and fourth week of life.¹¹ Therefore, we evaluated the effect of anticonvulsant treatment on survival during this vulnerable time window. *Scn1a*^{+/-} mice that underwent spontaneous seizure monitoring in either the naïve or hyperthermia-priming paradigms continued to receive anticonvulsant treatment through P30 to monitor survival (Fig. 7A, C). Carbamazepine ($P < 0.042$), levetiracetam ($P < 0.034$), and topiramate ($P < 0.042$) all prevented lethality, with 100% of mice surviving through P30. In contrast, only 67% (14/21) of untreated *Scn1a*^{+/-} mice survived to P30.

Because lamotrigine treatment resulted in such a profound worsening of seizure frequency, we hypothesized that lamotrigine would also negatively affect survival of *Scn1a*^{+/-} mice. We examined survival in a separate cohort of lamotrigine-treated and untreated *Scn1a*^{+/-} mice for an extended period (through P42). Surprisingly, although lamotrigine treatment significantly exacerbated the frequency of spontaneous GTCS, it significantly improved 6-week survival of *Scn1a*^{+/-} mice ($P < 0.015$). Lamotrigine treatment completely prevented lethality compared to only 68% (15/22) of untreated animals surviving to P42 (Fig. 7B).

Combination therapy

Stiripentol is approved for use as an add-on therapy in Dravet syndrome and is often combined with clobazam. We evaluated whether combination therapy with clobazam and stiripentol would offer additional therapeutic benefit compared to monotherapy. First, we tested the effect of acute combination of stiripentol (25 mg/kg, ip) with varying doses of clobazam on hyperthermia-induced seizure threshold in *Scn1a*^{+/-} mice. There was a left-shift in the dose-response curve with clobazam plus stiripentol compared to clobazam alone (EC₅₀ ratio = 0.7) (Fig. 8A). Next, we tested the effect of clobazam plus stiripentol therapy on spontaneous GTCS following hyperthermia-priming. Following induction of a single hyperthermic seizure, *Scn1a*^{+/-} mice were subchronically treated with

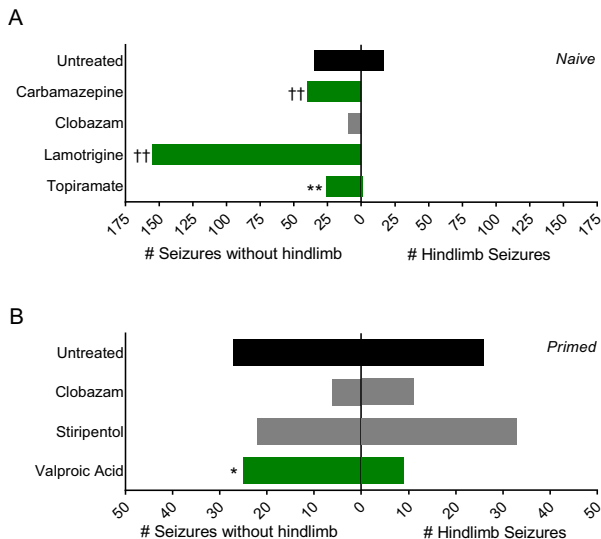


Figure 6. Anticonvulsant treatment alters spontaneous GTCS severity. (A) The total number of spontaneous GTCS with or without full tonic hindlimb extension of naïve untreated and drug-treated mice is displayed ($n = 8$ –17 mice per treatment group). The proportion of spontaneous GTCS without tonic hindlimb extension was significantly greater in carbamazepine, lamotrigine, and topiramate-treated mice (green bars) compared to untreated controls. (B) The total number of spontaneous GTCS with or without full tonic hindlimb extension of primed untreated and drug-treated mice is displayed ($n = 13$ –18 mice per treatment group). The proportion of spontaneous GTCS without tonic hindlimb extension was significantly less in valproic acid-treated mice (green bars) compared to untreated controls. Total number of spontaneous GTCS and seizure severity were scored over a 60-hour recording period. (* $P < 0.05$, ** $P < 0.01$, †† $P < 0.0001$, Fisher's exact test).

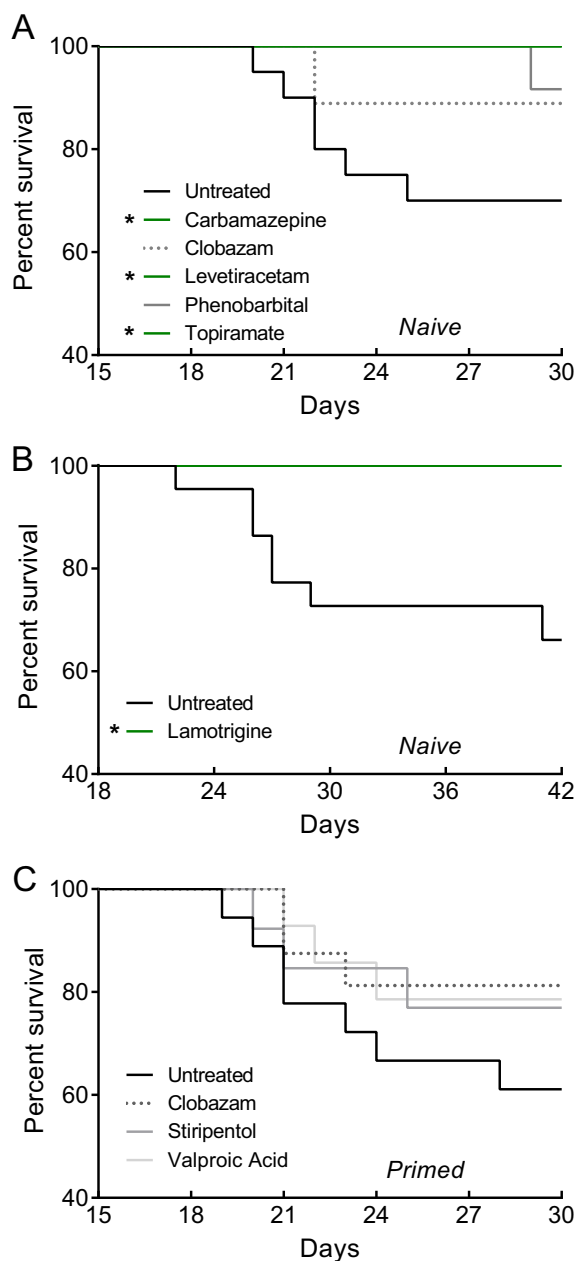


Figure 7. Survival analysis of *Scn1a*^{+/-} mice. Survival curves comparing untreated and drug-treated naïve (A, B) or hyperthermia-primed (C) *Scn1a*^{+/-} mice. Treatment began at P18 and survival was monitored until P30. (A) Carbamazepine, levetiracetam, and topiramate treatment (green lines) significantly improved survival compared to untreated naïve mice ($n = 10$ – 21 mice per treatment group). (B) Survival curves comparing untreated and lamotrigine-treated *Scn1a*^{+/-} mice. Treatment began at P18 and survival was monitored until P42 ($n = 15$ – 22 mice per treatment group). Lamotrigine treatment significantly improved survival compared to untreated mice (C). No difference in survival was identified in hyperthermia-primed mice ($n = 14$ – 19 mice per treatment group). (* $P < 0.05$, Logrank Mantel-Cox).

clobazam and stiripentol (160 mg per kg of chow and 4000 mg per kg of chow, respectively). Treatment with clobazam plus stiripentol provided significant protection against subsequent spontaneous GTCS in *Scn1a*^{+/-} mice, with 89% (17/19) seizure-free compared to 44% (11/25) of untreated mice ($P < 0.004$) (Fig. 8B). While there was a trend toward protection with clobazam alone, with 75%, seizure-free (12/16), it was not significantly different from untreated ($P > 0.062$) in this particular experiment. As expected, the addition of stiripentol increased the plasma concentration of both clobazam and *N*-desmethyl-clobazam in subchronically treated *Scn1a*^{+/-} mice (Fig. 8C).

Discussion

Approximately, 30 drugs are approved for the treatment of epilepsy; however, these drugs fail to provide adequate seizure control in approximately 30% of patients. Therefore, there is a critical need for the discovery of novel drugs for epilepsy. Most current anticonvulsant drug screening programs use induced seizures in wild-type animals to assess new therapeutic compounds. Genetic epilepsy models could provide alternative screening platforms. A frequently cited limitation of genetic models is low statistical power due to incomplete penetrance and low seizure frequency, which is consistent with our initial experience using spontaneous seizure monitoring in a 60-h time window. However, we found that adding an etiologically relevant hyperthermia-priming seizure was effective for improving statistical power to efficiently discriminate a treatment effect with reasonable group sizes.

In the context of monotherapy, we identified several anticonvulsant drugs (clobazam, valproic acid, stiripentol, levetiracetam, phenobarbital) that improved thermally induced and spontaneous seizure outcomes and correlated with our clinical observations or literature reports.^{5,7,32–34} Clobazam was the most effective drug for seizure reduction in the *Scn1a*^{+/-} mice, with a protective benefit against both spontaneous and thermally induced seizures at doses that produce plasma concentration levels within the human therapeutic range. This is consistent with the clinical experience from our Dravet syndrome cohort, where clobazam treatment resulted in seizure reduction in almost 75% of cases, and is the most consistently beneficial treatment reported in the literature.^{5,7,32–34} Previous studies in *Scn1a* Dravet mouse models reported beneficial effects with benzodiazepines, including clobazam and clonazepam.^{35,36} Conversely, lamotrigine produced significant elevation of spontaneous seizure frequency and lowered the threshold for thermally induced seizures in mice. Again, this is consistent with what is observed in our clinic, where lamotrigine treatment resulted in seizure worsening

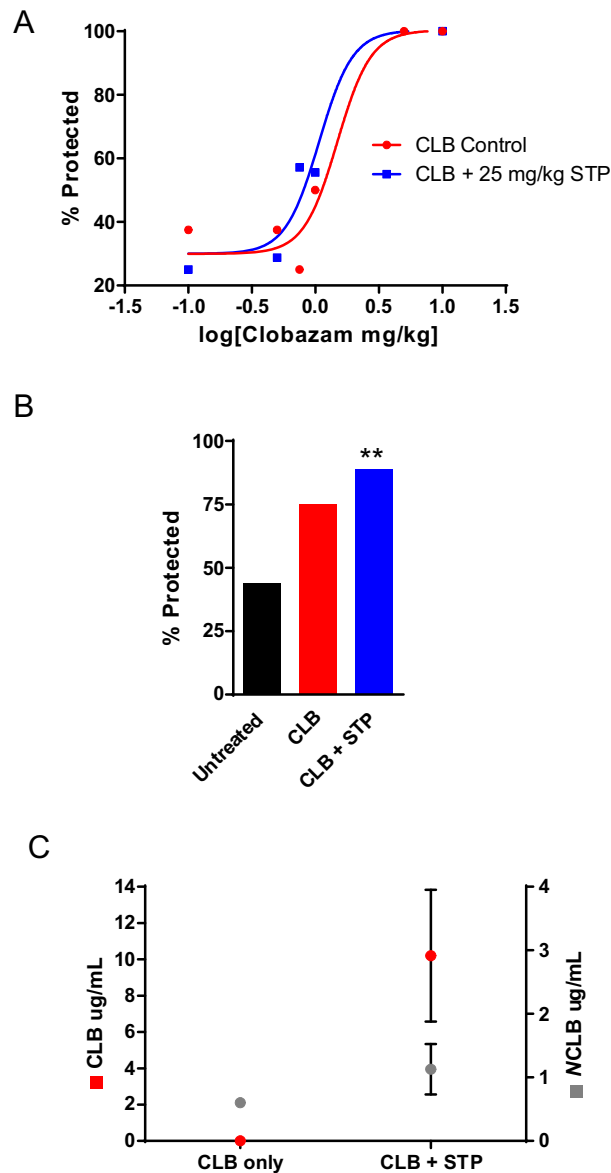


Figure 8. Combination therapy with clobazam and stiripentol in *Scn1a*^{+/-} mice. (A) Dose-response curve for seizure protection in hyperthermia-induced seizure threshold testing. *Scn1a*^{+/-} mice treated with varying doses of clobazam and 25 mg/kg of stiripentol resulted in a left-shift of the EC₅₀ compared to clobazam-only treated mice (*n* = 8–17 mice per treatment group). (B) The percentage of untreated, clobazam only, and clobazam plus stiripentol-treated mice that did not have spontaneous GTCS during the 60-hour recording period. Drug treatment was initiated following the induction of a single hyperthermia-induced seizure (*n* = 16–25 mice per treatment group). (C) Average clobazam and *N*-desmethylclobazam plasma concentrations of *Scn1a*^{+/-} mice treated subchronically with clobazam-only or clobazam plus stiripentol. The combination treatment resulted in higher clobazam and *N*-desmethylclobazam plasma levels (*n* = 6–7 mice per treatment group, error bars represent SEM). (***P* < 0.004).

in almost 50% of cases, and is consistent with literature reports.^{7,9} Although we observed a protective benefit with levetiracetam and phenobarbital in the *Scn1a*^{+/-} mice, these drugs had limited effectiveness in our patient series. However, there are reports of favorable response with these drugs in other Dravet syndrome patient series and a recent consensus panel recommends levetiracetam and phenobarbital as third line treatments.^{5,7,34} Although phenobarbital was one of the best performers in the *Scn1a*^{+/-} mice, its use as a maintenance therapy in patients is often limited by the perceived long-term side effects. Therefore, the Dravet syndrome patient response data are based on short-term use, mainly in infants.

Patients with drug-resistant epilepsy, like Dravet syndrome, are most often on polytherapy. The average number of AEDs per patient in our *SCN1A*-mutation-positive cohort was 3 (SD 1). Although monotherapy in the mice allowed us to individually assess the effect of each drug, it did not account for drug–drug interactions that may contribute to efficacy or adverse effects. The only drug specifically indicated for Dravet syndrome, stiripentol, was approved as an add-on treatment and is most often combined with clobazam and/or valproic acid. In the *Scn1a*^{+/-} mice, stiripentol was effective at high doses in our hyperthermia assay, but was ineffective in all assays when using a dose that targeted the human therapeutic range. Consistent with this, intrinsic anticonvulsant activity has previously been reported for stiripentol in various rodent models, including with *Scn1a*^{+/-} Dravet mice, with high doses, resulting in plasma levels 8–10-fold higher than the human therapeutic range.^{35,37,38} It has been hypothesized that stiripentol derives some of its therapeutic benefit from synergistic effects with other anticonvulsant drugs, especially clobazam. In our study, combination therapy in *Scn1a*^{+/-} mice using a clinically relevant dose of stiripentol with varying clobazam doses resulted in a shift in the dose-response curve in the hyperthermia-induced seizure threshold assay, and subchronic combination therapy increased the percent protected from spontaneous GTCS following hyperthermia-priming. Stiripentol is a weak allosteric modulator of GABA_A receptors, a shared mechanism with clobazam, and a strong inhibitor of cytochrome P450 enzymes, particularly CYP2C19. Inhibition of CYP2C19 by stiripentol can promote higher plasma levels of clobazam, *N*-desmethylclobazam, and valproic acid in patients on polytherapy.^{39,40} Consistent with reports of cytochrome P450 inhibition by stiripentol, we observed significant elevation of plasma clobazam concentration following subchronic combination therapy with clobazam plus stiripentol in *Scn1a*^{+/-} mice relative to clobazam monotherapy (Fig. 8C).

Most surprising to us was the improved survival observed with some drugs that had no protective effect on spontaneous seizures or hyperthermia-induced seizure threshold. This was most evident with lamotrigine, as treatment worsened seizure frequency but completely prevented the early lethality of *Scn1a*^{+/-} mice. This paradoxical effect may be due to the lower severity of individual seizures during lamotrigine exposure, or may result from protective action of lamotrigine outside the central nervous system. Nevertheless, it highlights the fact that survival may not be the most reliable predictor of therapeutic anticonvulsant activity in this model.

An important aspect of this study was our incorporation of pharmacokinetics to benchmark our doses relative to the human therapeutic range. Comprehensive pharmacokinetic profiling in rodent studies is crucial to aid in the translation of preclinical data to human response. Kleiman and Ehlers suggest that a lack of proper pharmacokinetic data and consideration during preclinical experimental design may be hindering the ability to extrapolate results into a clinical setting.⁴¹ In our study, we evaluated anticonvulsant doses that resulted in plasma concentrations within the human therapeutic range to ensure an accurate assessment of relevant drug responses in our model. Use of clinically relevant exposure levels mirrors the clinical response more closely, for example, in our hyperthermia assay, lower doses of valproic acid and clobazam provide some therapeutic benefit but not seizure freedom, while escalation of dose beyond the therapeutic range resulted in complete protection from seizures. This is consistent with human data, where valproic acid and clobazam often reduce seizure frequency, but rarely result in seizure freedom. Overall, our findings demonstrate the importance of pharmacokinetic profiling for interpretation of preclinical epilepsy therapy studies and suggest a potential source of the failure to translate some preclinical studies to efficacy in humans where dose range may be limited by safety concerns.

Recently, the *scn1Lab*-mutant zebrafish model was demonstrated to recapitulate features of Dravet syndrome and has been used for anticonvulsant screening.^{42,43} The large-scale nature of in vivo phenotype-based screening in *scn1Lab* zebrafish could substantially increase the identification of compounds with anticonvulsant potential. Although compounds that already have safety data and regulatory approval may go directly to patient trials, novel development compounds will need to be validated in mammalian model systems.⁴⁴ Here, we have demonstrated that the *Scn1a*^{+/-} mouse model of Dravet syndrome recapitulates clinical pharmacological responses. A platform coupling high-throughput screening in the *scn1Lab* zebrafish with validation studies in *Scn1a*^{+/-} mice could be synergistic and

advance testing of novel anticonvulsant compounds in genetic models.

Overall, our study suggests that the most predictive measures for screening new compounds in the *Scn1a*^{+/-} Dravet mouse model would be thorough evaluation of dose-response with hyperthermia-induced seizures and spontaneous seizure frequency monitoring after hyperthermia-priming in *Scn1a*^{+/-} mice. Our study provides a foundation to predict the efficacy of novel compounds in the treatment of Dravet syndrome and suggests useful strategies for development of preclinical screenings in other genetic models of pharmacoresistant epilepsies.

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Author Contributions

Conception and design of the study: JAK and ALG. Acquisition and analyses of data: NAH, LLA, TSG, LL. All authors contributed to drafting the manuscript or figures: NAH, LLA, TSG, LL, ALG, and JAK.

Conflicts of Interest

The authors declare no competing interests.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Conditions for AED high performance liquid chromatography (HPLC) assays

Figure S1. *Pharmacokinetic analysis of stiripentol and clobazam.* Plasma concentration-time curves for A. stiripentol (following 100 mg/kg, ip injection) and B. clobazam (CLB) and *N*-desmethyloclobazam (NCLB) (following 10 mg/kg clobazam, ip injection). Data represent average plasma concentrations from $n = 3–5$ mice per time point.

Figure S2. *Chromatograms from HPLC analysis.* Representative chromatograms of plasma samples spiked with A. carbamazepine and lamotrigine B. phenobarbital C. stiripentol D. clobazam E. *N*-desmethyloclobazam F. levetiracetam and respective internal standards.

Video S1. *Untreated *Scn1a*^{+/-} GTCS.* Representative example of an untreated *Scn1a*^{+/-} mouse experiencing a spontaneous GTCS with tonic hindlimb extension. The seizure begins with rearing and paddling, quickly with loss of posture, rapid running and jumping, and ending in hindlimb extension.

Video S2. *Lamotrigine-treated *Scn1a*^{+/-} GTCS.* Representative example of a lamotrigine-treated *Scn1a*^{+/-} mouse experiencing a spontaneous GTCS. The seizure begins with rearing and paddling, followed by loss of posture, rapid running and jumping, and ending with vigorous clonic movements of fore- and hindlimbs while the mouse lay on its side, spinning in circles.

Video S3. *Carbamazepine-treated *Scn1a*^{+/-} GTCS.* Representative example of a carbamazepine-treated *Scn1a*^{+/-} mouse experiencing a spontaneous GTCS. The seizure begins with several myoclonic jerks and spinning in circles, before progressing to GTCS with loss of posture, rearing and paddling, but no tonic hindlimb extension.

Video S4. *Topiramate-treated *Scn1a*^{+/-} GTCS.* Representative example of a topiramate-treated *Scn1a*^{+/-} mouse experiencing a spontaneous GTCS. The seizure begins with rearing and paddling and progresses without loss of posture or tonic hindlimb extension.