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FULL-LENGTH ORIGINAL RESEARCH

Development of an antiseizure drug screening platform for Dravet syndrome at the NINDS contract site for the Epilepsy Therapy Screening Program

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Abstract

Objective: Dravet syndrome (DS) is a rare but catastrophic genetic epilepsy, with 80% of patients carrying a mutation in the *SCN1A* gene. Currently, no antiseizure drug (ASD) exists that adequately controls seizures. In the clinic, individuals with DS often present first with a febrile seizure and, subsequently, generalized tonic-clonic seizures that can continue throughout life. To facilitate the development of ASDs for DS, the contract site of the National Institute of Neurological Disorders and Stroke (NINDS) Epilepsy Therapy Screening Program (ETSP) has evaluated a mouse model of DS using the conditional knock-in *Scn1a*$^{A1783V/WT}$ mouse.

Methods: Survival rates and temperature thresholds for *Scn1a*$^{A1783V/WT}$ were determined. Prototype ASDs were administered via intraperitoneal injections at the time-to-peak effect, which was previously determined, prior to the induction of hyperthermia-induced seizures. ASDs were considered effective if they significantly increased the temperature at which *Scn1a*$^{A1783V/WT}$ mice had seizures.

Results: Approximately 50% of *Scn1a*$^{A1783V/WT}$ survive to adulthood and all have hyperthermia-induced seizures. The results suggest that hyperthermia-induced seizures in this model of DS are highly refractory to a battery of ASDs. Exceptions were clobazam, tiagabine, levetiracetam, and the combination of clobazam and valproic acid with add-on stiripentol, which elevated seizure thresholds.

Significance: Overall, the data demonstrate that the proposed model for DS is suitable for screening novel compounds for the ability to block hyperthermia-induced seizures and that heterozygous mice can be evaluated repeatedly over the course of several weeks, allowing for higher throughput screening.

KEYWORDS

genetics, hyperthermia-induced seizures, mouse model, voltage-gated sodium channel

1 | INTRODUCTION

Dravet syndrome (DS) is a rare, catastrophic form of genetic epilepsy that manifests in the first year of life of a seemingly normal infant.[1] In 80% of cases, the disease is caused by a mutation in the *SCN1A* gene, which encodes for the voltage-gated sodium channel Nav1.1.[2] There are over 900 distinct *SCN1A* mutations, with a large percent resulting from missense or frameshift mutations, leading to a loss of function in the sodium channel.[3] In mouse models of DS, the *Scn1a* mutation reduces sodium currents and excitability in inhibitory interneurons.[4–7] As a result, this causes an imbalance between inhibition and excitation, leading to general hyperexcitability. Seizures in individuals with DS are usually first induced by a fever or other increases in core temperature.[2] Spontaneous seizures typically begin within weeks of the initial seizure, becoming progressively worse and occurring more frequently.[8] In addition to high seizure burden, DS also negatively impacts development and behavior. Furthermore, DS is associated with a higher rate of sudden unexpected death in epilepsy (SUDEP). DS is highly pharmacoresistant, with the primary treatment goal to decrease the seizure frequency and prevent status epilepticus.[9] Current treatment options fail to adequately address both seizures and the comorbidities associated with DS in many patients; therefore, discovering effective anti-seizure drugs (ASDs) is imperative. Typical first-line treatments for DS include valproate and clobazam, whereas stiripentol and topiramate are secondary lines of treatment, usually used congruently with valproate and clobazam.[10–12] Both cannabidiol and fenfluramine are promising new therapeutics in patients with DS, with cannabidiol reducing motor seizures by ~40%[13,14] and fenfluramine reducing seizures by ~50%.[15–17] Some commonly used ASDs for seizure control, such as carbamazepine and lamotrigine, exacerbate seizures in both children and mice with DS conferring mutations.[8,18] These sodium channel blockers worsen seizures in patients due to reduced SCN1A function in inhibitory neurons, thereby promoting hyperexcitability.[8] Although there have been recent advances in treatment options for patients with DS, full seizure freedom has yet to be achieved. Clinically relevant animal models with good face, construct, and predictive validity are critically needed to successfully screen and discover effective, novel therapies.

An important goal at the National Institute of Neurological Disorders and Stroke (NINDS) contract site for the Epilepsy Therapy Screening Program (ETSP) is to have well-characterized, reliable, and translatable models to facilitate the development of novel therapies for the treatment of epilepsy, including genetic epilepsies such as DS. To develop a screening model for DS for potential adoption by the program, mice with a mutation in *Scn1a* were obtained from Jackson Laboratories. The mutation mini-cassette includes lox P sites and, in the presence of Cre recombinase, results in an amino-acid substitution from alanine to valine at position 1783 (A1783V), reported previously in patients with DS.[19,20] This mutation in mice results in both hyperthermia-induced seizures, which mimics febrile seizures seen in a clinical setting, and spontaneous, recurrent seizures.[21–23] In the present study, we show the potential of using the hyperthermia-induced seizure phenotype to rapidly screen novel therapeutics for the treatment of DS. Previous data have pharmacologically profiled a knock-out model of DS using the hyperthermia-induced seizure model.[18] However, with more than 900 mutations resulting in DS, and 47% of these resulting from a missense mutation, it is important to have a number of models characterized to address the diverse patient population.[24] To characterize this mouse model, a survival analysis and a temperature threshold at which heterozygous (*Scn1a*$^{A1783V/WT}$) mice seize was determined. Following these experiments, a battery of ASDs was administered to identify the pharmacological characteristics of this preclinical model, which demonstrated pharmacoresistance to many available treatments. These data will serve as a metric for the identification of novel anti-seizure compounds.

Key Points

- *Scn1a*$^{A1783V/WT}$ mice have a 50% survival rate, and all have hyperthermia-induced seizures.
- Common DS treatments such as clobazam (CLB) and combinatorial therapy of CLB, valproic acid (VPA), and stiripentol (STP) increase temperature thresholds in *Scn1a*$^{A1783V/WT}$ mice.
- Sodium channel blockers, such as carbamazapine (CBZ) and lamotrigine (LTG), decrease temperature thresholds of *Scn1a*$^{A1783V/WT}$ mice as predicted.
- Hyperthermia-induced seizures in *Scn1a*$^{A1783V/WT}$ mice are highly pharmacoresistant to common ASDs.
- The *Scn1a*$^{A1783V/WT}$ may be a useful preclinical drug screening platform for the treatment of DS.

2 | METHODS

2.1 | Animals

All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Utah. Animal experiments were conducted in a manner consistent with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines (https://www.nc3rs.org.uk/arrive-guidelines). Experimental animals

were generated by breeding a floxed stop male *Scn1a*^{A1783V} (B6(Cg)-Scn1atm1.1Dsf/J, Jax #026133) with a Sox2-cre (B6. Cg-Edil3 $^{Tg(Sox2-cre)1Amc}$/J) female mouse to produce both heterozygous (*Scn1a*$^{A1783V/WT}$) and wild-type offspring. Both female and male heterozygous and age-matched wild-type littermates were used for experiments (4–6 weeks of age). Mice were group housed in a pathogen-free facility under a 12-h light/12-h dark-light cycle and had access to food and water ad libitum, except during hyperthermia-induced seizure experiments, in which case they were transferred to the experimental room approximately 1 hour before testing.

2.2 | Hyperthermia-induced seizures

To evaluate the temperature at which *Scn1a*$^{A1783V/WT}$ mice seize, mice were placed under a heat lamp and core temperature was gradually raised in an acrylic glass chamber until a generalized seizure was observed or the temperature reached 42.5°C.[18,25–27] After the procedure, mice were transferred to a cool, granite block to rapidly lower the core temperature, and then they were returned to their home cages. Body temperature was monitored using a neonate rectal probe (Braintree Scientific, Inc, Braintree, MA) coupled to a TCAT-2LV controller (Physitemp Instruments, Inc). Mice acclimated in the chamber for 5 minutes prior to experiments. Male and female *Scn1a*$^{A1783V/WT}$ randomly received drug or vehicle prior to the test and staff were blinded to the treatment. If a mouse had a behavioral seizure between drug administration and testing, it was removed from the study. The temperature at which mice seized was recorded. In experiments where wild-type mice were used, the staff was also blinded to genotype. Average baseline temperatures and the number of male and female mice used for each compound tested are shown in Table S1, with minimal variability between tests. The rate of temperature change (temperature (°C)/time to seizure (s)) for each drug tested is also shown in Table S2.

2.3 | Drug preparation and administration

Carbamazepine, clobazam, clonazepam, phenobarbital, phenytoin, stiripentol, and valproic acid were purchased from Sigma. Levetiracetam, lamotrigine, tiagabine, topiramate, fenfluramine, and rufinamide were obtained from TCI America. Cannabidiol was purchased from Cayman Chemical. Ezogabine and lacosamide were purchased from Axon Medchem. For acute administration, all drugs were prepared as 0.5% methyl cellulose (Sigma) suspensions, as this is a good universal solvent and kept the vehicle consistent between tests. All drugs were administered in a volume of 0.01 mL/g. The dose (mg/kg) for each drug is listed in Table 1. All drug compounds were administered and tested based on their time-of-peak effect (TPE), which was determined previously in the maximal electroshock seizure (MES) model or in the 6 Hz test (data not shown). Intraperitoneal (i.p.) injections were administered at the TPE for each drug, which are listed in Table 1, prior to testing. For 5-day sub-chronic administration, compounds were administered at the same time each day and, on the last day, was administered at the TPE prior to hyperthermia-induced seizure testing. For the triple therapy study, clobazam and stiripentol were administered at 1.0 hour as a single injection and valproic acid was administered 0.25 hour in a separate injection prior to testing. In all testing, experimenters were blinded to treatment group and mice were randomly assigned to treatment group.

2.4 | Cross-over studies

For cross-over studies, an initial cohort of 4-week-old *Scn1a*$^{A1783V/WT}$ mice was randomly assigned into two to three treatment groups, including one vehicle group. The following week, mice were crossed over to another treatment group. This process was repeated for a maximum of 4 weeks (Figure S1). At the completion of testing, each mouse had received all treatments. The cross-over design is used to minimize the number of mice needed for experiments. For each cross-over experiment, each mouse was tested with every drug, including vehicle. Each mouse was subjected to a hyperthermia-induced seizure a maximum of four times and this was consistent for each mouse in the cross-over experiment. Drugs that were included in cross-over experiments were tested weekly.

2.5 | Statistical analysis

Statistical comparisons were conducted using a Log-rank (Mantel-Cox) test. A power analysis for the log-rank (Mantel-Cox) test was conducted using $\alpha = .05$ and the hazard ratio = 0.20. To detect differences with a power of 80%, a total sample size of $N = 14$ is required for each compound tested. Significance was defined as a p-value $<.05$. All analysis was conducted with GraphPad Prism 8.0. Sample sizes and statistical results are reported in figure legends. Data are presented as mean ± standard deviation (SD).

3 | RESULTS

3.1 | Female *Scn1a*$^{A1783V/WT}$ mice exhibit greater mortality than male mice during development

The *Scn1a*$^{tm1.1Dsf}$ mouse has a conditional knock-in mutation that is expressed when exposed to cre recombinase. For all

TABLE 1 List of prototype drugs evaluated in *Scn1a*$^{A1783V/WT}$ mice following intraperitoneal administration

Drug	Dosing strategy	Dose (mg/kg)	TPE (h)
Clobazam (CLB)	Single	2.5, 5, 10	1.0
Carbamazepine (CBZ)	Single	40	0.25
Cannabidiol (CBD)	Single	100, 200	1.0
Clonazepam (CLN)	Single	0.5	0.5
Ezogabine (EZG)	Single	20	1.0
Fenfluramine (FFA)	Single	10, 25	0.5
Fenfluramine (FFA)	Single	10, 25	4.0
Lacosamide (LCM)	Single	15	0.5
Lamotrigine (LTG)	Single, sub-chronic	10, 20	1.0
Levetiracetam (LEV)	Single	100, 500	1.0
Lorcaserin	Single	10	0.5
Phenobarbital (PHB)	Single	15	0.5
Retigabine	Single	20	1.0
Rufinamide	Single	32	0.5
Stiripentol (STP)	Single	100	1.0
Tiagabine (TGB)	Single	1.3, 3, 5	1.0
Topiramate (TPM)	Single	300	1.0
Valproic Acid (VPA)	Single	300	0.25
CLB + VPA	Single	5, 150	1.0, 0.25
CLB + VPA	Single	5, 75	1.0, 0.25
CLB + VPA + STP	Single	5, 75, 100	1.0, 0.25, 1.0
CLB + VPA + STP	Single	5, 75, 30	1.0, 0.25, 1.0

experiments, *Scn1a*$^{tm1.Dsf}$ male mice were bred to female *Sox2-cre* mice. As a result of cre expression in oocytes, the offspring are either heterozygous for the *Scn1a* mutation (*Scn1a*$^{A1783V/WT}$) or wild-type (WT). The Sox2-cre mouse line express Cre recombinase under the control of the Sox2 promoter and is useful for generating epiblast-derived specific conditional mutations. Breeding these mice with mice containing loxP-flanked sequences, will result in the deletion of the floxed sequences in Sox2-expressing tissue in the offspring due to cre-mediated recombination. At embryonic day 6.5, activity is detected in epiblast cells. Transgene expression is active in the female germline and offspring will exhibit Cre activity, independent of the genotype. This effect from the female germline expression of the transgene can provide a quick and efficient breeding mechanism for generating experimental mice.[28] Both the heterozygous and WT offspring were assessed for survival and compared. Over 60 days, ~50% of the *Scn1a*$^{A1784V/WT}$ mice survived, whereas no deaths were observed in WT littermates (Figure 1A). When survival was evaluated based on sex, male *Scn1a*$^{A1783V/WT}$ mice had a significantly higher survival rate (60.0%) than female mice (32.0%) by adulthood (Figure 1B).

3.2 | Male and female *Scn1a*$^{A1783V/WT}$ mice have similar temperature thresholds for hyperthermia-induced seizures

The temperature threshold for which both male and female *Scn1a*$^{A1783V/WT}$ mice seize was determined by subjecting mice to a steady increase in core temperature. Testing was discontinued when core temperature reached 42.5°C.[18,26,27] If no seizure occurred by 42.5°C, the mouse was considered seizure-free. All evaluated *Scn1a*$^{A1783V/WT}$ mice had hyperthermia-induced seizures, with an average temperature threshold of 38.5 ± 1.9°C. There was no evidence of seizure activity when the core temperature of wild type littermates was raised to 42.5°C (Figure 1C). To further evaluate if sex-dependent temperature thresholds exist, data were evaluated by sex. There was no significant difference between temperature thresholds for male (38.6 ± 1.8°C) and female (38.7 ± 1.7°C) *Scn1a*$^{A1783V/WT}$ mice (Figure 1D). As a result, both male and female mice were used for testing.

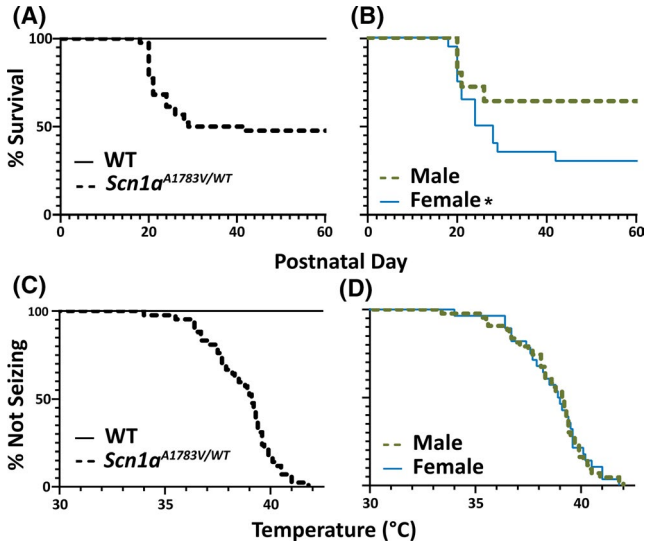

FIGURE 1 Approximately 50% of *Scn1a*$^{A1783V/WT}$ mice survive to adulthood and female *Scn1a*$^{A1783V/WT}$ mice exhibit greater mortality than male mice during development. (A) By postnatal day 60, 51.1% of the observed *Scn1a*$^{A1783V/WT}$ mice die, with the first death occurring at or around the age at which mice are weaned (P21). None of the WT offspring died during the 60-day observation period (*Scn1a*$^{A1783V/WT}$: $n = 45$, WT: $n = 30$). (B) When survival was evaluated based on sex, males had a significantly lower death rate (32.0%) than females (60.0%) by day 60 (males: $n = 25$, females: $n = 20$, $p = .0397$, Log-rank [Mantel-Cox]). (C) All *Scn1a*$^{A1783V/WT}$ mice had hyperthermia-induced seizures while age-matched WT littermates had no evidence of seizure activity (*Scn1a*$^{A1783V/WT}$: $n = 71$, WT: $n = 16$). (D) There was no significant difference between the temperatures at which male and female *Scn1a*$^{A1783V/WT}$ mice seized (males: $n = 43$, females: $n = 28$, $p = .959$, Log-rank [Mantel-Cox])

3.3 | *Scn1a*$^{A1783V/WT}$ mice can be used for several weeks of drug screening

To determine if *Scn1a*$^{A1783V/WT}$ mice continue to have hyperthermia-induced seizures as they age and if mice could be used over the course of several weeks for drug screening, two compounds and vehicle were administered to the same mice over the course of 4 weeks. Mice were divided randomly into three initial treatment groups (vehicle, clobazam (CLB) [10 mg/kg], and carbamazepine (CBZ) [40 mg/kg]). At each week, CLB, a drug that reduces seizure frequency in patients with DS,[10] significantly increased the temperature at which *Scn1a*$^{A1783V/WT}$ mice had hyperthermia-induced seizures as compared to both vehicle- and CBZ-treated mice. On the other hand, CBZ, a drug that worsens seizures in patients with DS,[10] significantly decreased the temperature threshold at which *Scn1a*$^{A1783V/WT}$ mice seized each week (Figure 2). Repeat treatment and repeat hyperthermia-induced seizures did not significantly affect the temperatures at which mice seized each week (Figure S2).

3.4 | Evaluation of prototype ASDs against hyperthermia-induced seizures

Additional ASDs that have previously shown efficacy in the treatment of DS, or those that are known to worsen seizure outcomes, were evaluated against hyperthermia-induced seizures to determine the predictive validity of the *Scn1a*$^{A1783V/WT}$ mouse model. Of those drugs known to confer seizure protection in DS, both CBD and FFA were evaluated.[13,29,30] CBD (200 mg/kg) did not significantly increase the temperature at which hyperthermia-induced seizures occurred (Table 2). FFA was evaluated at two doses, 10 mg/kg and 25 mg/kg and two different TPEs, 0.5 h and 4 h. Neither dose nor TPE had a significant effect on temperature threshold in *Scn1a*$^{A1783V/WT}$ mice (Figure S3A-D, Table 2).

Sodium channel blockers such as CBZ and LTG can increase the frequency or severity of seizures in patients with DS.[10] CBZ, 40 mg/kg, significantly lowered the temperature at which hyperthermia-induced seizures occurred (Figure 2A-D). LTG was tested as a single 10 mg/kg dose and had no effect on the temperature threshold (Figure S3E, Table 2). However, when 20 mg/kg LTG was sub-chronically administered daily for 5 days and a hyperthermia-induced seizure was conducted on Day 5, a significantly lowered temperature threshold as compared to vehicle-treated mice was observed. In addition, during the 5-day administration of LTG, two of the six mice in the treatment group died (Figure S3F).

3.5 | Except for tiagabine and high-dose levetiracetam, hyperthermia-induced seizures in *Scn1a*$^{A1783V/WT}$ mice are refractory to a battery of anti-seizure drugs

In addition to the compounds evaluated above, other prototype ASDs used in the treatment of epilepsy were evaluated. Of these additional compounds, only tiagabine (TGB) was found to be effective in shifting the temperature threshold at which seizures occurred. TGB, which has shown to be protective against hyperthermia-induced seizures in a haploinsufficient mouse model of DS,[31] was evaluated at 1.3, 3, and 5 mg/kg in a cross-over scheme. At all doses, TGB significantly increased the temperature at which mice seized as compared to vehicle (Figure 3A-C), and there was no significant difference between vehicle-treated mice for each dose (Figure S4). Although a dose of 100 mg/kg levetiracetam (LEV) (Figure 3D) did not confer significant protection, a higher dose (500 mg/kg) of LEV did significantly increase the temperature at which mice seized (Figure 3E). None of the other ASDs listed in Table 2 significantly affected the temperature threshold at which *Scn1a*$^{A1783V/WT}$ mice had a hyperthermia-induced seizure ($p > .05$, Log-rank [Mantel-Cox]), suggesting that hyperthermia-induced seizures in

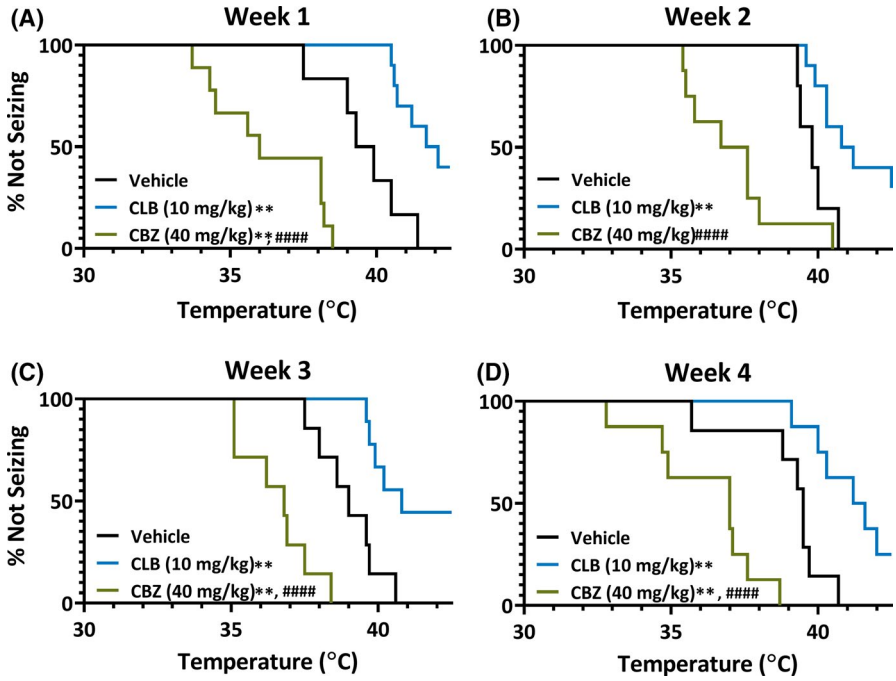

FIGURE 2 Repeat testing does not change pharmacological outcome of seizures in *Scn1a*$^{A1783V/WT}$ mice. *Scn1a*$^{A1783V/WT}$ mice were divided randomly into three initial treatment groups (vehicle, clobazam (CLB), and carbamazepine (CBZ)). Each week, mice were given the same treatment. At each week, CLB significantly increased the temperature threshold for which mice had hyperthermia-induced seizures when compared to vehicle-treated mice (Week 1, $p = .0013$; Week 2, $p = .0076$; Week 3, $p = .0027$; Week 4, $p = .0015$; Log-rank [Mantel-Cox]). CLB also conferred complete protection in three mice at Week 1, in three mice at Week 2, four mice at Week 3, and two mice at Week 4. Alternatively, CBZ decreased the temperature threshold each week, with a significant reduction seen at Weeks 1, 3, and 4 (Week 1, $p = .0022$; Week 2, $p = .051$; Week 3, $p = .0014$; Week 4, $p = .0053$ vs vehicle; Log-rank [Mantel-Cox]). At each week, CLB significantly increased the temperature threshold as compared to CBZ (Week 1–4, Week 3: $p < .0001$, Log-rank [Mantel-Cox]). Vehicle: Week 1, $n = 6$; Week 2, $n = 4$; Week 3, $n = 7$; Week 4, $n = 7$ and CLB: Week 1, $n = 10$; Week 2, $n = 10$; Week 3, $n = 9$; Week 4, $n = 8$; CBZ: Week 1, $n = 9$; Week 2, $n = 8$; Week 3, $n = 7$; Week 4, $n = 8$; $^{**}p < .01$, $^{***}p < .001$ vs vehicle, $^{\#}p < .0001$ vs CLB

this mouse model of DS are highly refractive to a battery of ASDs.

3.6 | Evaluation of add-on treatment against hyperthermia-induced seizures

First-line treatment of DS includes either CLB or VPA.[32] When either fails as independent treatment, CLB and VPA are administered together.[11] Stand-alone treatment of either 5 mg/kg CLB or 300 mg/kg VPA did not offer significant protection against hyperthermia-induced seizures in *Scn1a*$^{A1783V/WT}$ mice (Table 2). Therefore, as done in the clinic, CLB and VPA were administered congruently. CLB (5 mg/kg) and VPA (150 mg/kg) significantly increased the temperature threshold compared to vehicle-treated mice (Figure 4A). If this combination fails in the clinic, a relevant second-line drug regimen combines CLB and VPA with STP as an add-on drug.[11] We tested a combination of 5 mg/kg CLB and 75 mg/kg VPA, which did not offer significant protection (Figure 4B). To mimic what is done in the clinic, 100 mg/kg STP was then "added on" to 5 mg/kg CLB and 75 mg/kg VPA treatment. The CLB (5 mg/kg) + VPA (75 mg/kg) + STP (100 mg/kg) treated mice had significantly higher temperature thresholds than CLB (5 mg/kg) + VPA (75 mg/kg) treated *Scn1a*$^{A1783V/WT}$ mice (Figure 4C). Finally, we show that a lower dose of add-on STP did not increase temperature thresholds significantly when compared to the combination of CLB (5 mg/kg) and VPA (75 mg/kg) (Figure 4D). Thus this add-on screening approach may prove useful when screening novel compounds for efficacy against hyperthermia-induced seizures.

4 | DISCUSSION

Drug-resistant epilepsies, like DS, critically need preclinical drug-screening models to identify effective compounds. More than 900 distinct mutations result in this highly complex encephalopathy, further necessitating the availability of translatable models to discover effective therapies. Introducing new screening models to the ETSP requires understanding of both the face and predictive validity of proposed models. The *Scn1a*$^{A1783V/WT}$ model has a survival rate of ~50% (Figure

TABLE 2 Anti-seizure drugs refractory to hyperthermia-induced seizures in *Scn1a*$^{A1783V/WT}$ mice

Compound	Dose (mg/kg)	Vehicle Temp (°C)	Drug Temp (°C)	Efficacy (# Protected/*N*)	Significance (Log-rank)
Clonazepam	0.5	39.1 ± 2.0, $n = 10$	40.0 ± 1.7, $n = 10$	0/10	$p = .2385$
Phenobarbital	15	38.6 ± 1.6, $n = 4$	39.3 ± 1.0, $n = 6$	0/6	$p = .5789$
Lacosamide	15	37.5 ± 2.0, $n = 3$	37.1 ± 1.4, $n = 10$	0/10	$p = .4098$
Retigabine[a]	20	38.8 ± 1.9, $n = 10$	38.7 ± 1.7, $n = 10$	0/10	$p = .8593$
Rufinamide[a]	32	39.2 ± 1.5, $n = 10$	39.4 ± 1.5, $n = 9$	0/10	$p = .8846$
Topiramate[a]	300	39.2 ± 1.5, $n = 10$	39.3 ± 1.7, $n = 9$	0/10	$p = .7416$
Lorcaserin	10	37.9 ± 2.0, $n = 10$	37.6 ± 2.4, $n = 10$	0/10	$p = .6089$
Clobazam[a]	2.5	38.7 ± 1.0, $n = 7$	39.6 ± 0.7, $n = 9$	0/9	$p = .1212$
	5	38.7 ± 0.4, $n = 7$	39.8 ± 1.3, $n = 8$	1/8	$p = .0564$
Stiripentol[a]	100	38.2 ± 1.2, $n = 16$	37.8 ± 1.3, $n = 16$	0/16	$p = .849$
Valproic acid[a]	300	39.5 ± 1.2, $n = 11$	40.2 ± 1.0, $n = 11$	1/11	$p = .4677$
Cannabidiol	200	39.0 ± 1.5, $n = 10$	38.6 ± 2.0, $n = 10$	1/10	$p = .8119$
Fenfluramine[a]	10	39.0 ± 1.2, $n = 9$	39.0 ± 2.3, $n = 10$	0/10	$p = .4862$
	25	39.0 ± 0.7, $n = 10$	38.8 ± 2.1, $n = 10$	0/10	$p = .2480$
Fenfluramine[a], TPE 4 h	10	38.3 ± 1.3, $n = 6$	38.7 ± 0.5, $n = 7$	0/7	$p = .6526$
	25	36.7 ± 0.9, $n = 6$	37.8 ± 1.4, $n = 6$	0/6	$p = .0966$
Lamotrigine[a]	10	37.9 ± 2.0, $n = 9$	37.1 ± 1.3, $n = 9$	0/10	$p = .1898$
	20, sub	39.4 ± 0.8, $n = 6$	37.6 ± 0.9, $n = 4$	0/10	$p = .0046$

Note: Data presented as mean ± SD. Log-rank (Mantel-Cox).

[a]Indicates drug was tested in a cross-over scheme.

1A,B), demonstrating that the *Scn1a*$^{A1783V/WT}$ mouse model has at least as good or better viability than other models, increasing the yield of experimental mice.[4,22,33] *Scn1a*$^{A1783V/WT}$ mice maintain the hyperthermia-induced seizure phenotype (Figure 1C,D) as they age (Figure 2). Although males have a significantly higher survival rate than females (Figure 1B), there was no difference in the temperatures at which they seized (Figure 1D). The breeding scheme used here allows for approximately half the offspring to be heterozygous for the mutation. This allows for faster breeding than in other models, especially because the breeding pairs, who do not express the floxed transgene, survive readily. The predictive validity of this model should be well characterized prior to screening novel compounds, as this provides a baseline metric from which to determine if novel compounds have superior efficacy over available compounds. Clinically approved ASDs for the treatment of DS, ASDs that are contraindicated, as well as a battery of other ASDs, were screened to pharmacologically profile the *Scn1a*$^{A1783V/WT}$ mouse model.

A first-line treatment for DS is CLB.[10] We found that 10 mg/kg CLB significantly increased the temperature at which *Scn1a*$^{A1783V/WT}$ mice seized (Figure 2). CLB has also been tested against hyperthermia-induced seizures in the *F1.Scn1a*^{tm1Kea} mouse, and in that study, 5 mg/kg CLB conferred protection.[18] In another study, single injections of CLB, at 1 mg/kg and 10 mg/kg, both significantly increased the temperature threshold of the *F1.Scn1a*^{tm1Kea} mouse.[27] In a mouse model with a truncation mutation, *Scn1a*^{R1407X}, 6.62 mg/kg CLB also significantly increased the temperature at which mice had hyperthermia-induced seizures.[34] Overall, CLB has been effective against spontaneous seizures in patients[32] and against hyperthermia-induced seizures in, along with the data obtained herein, three DS mouse models.

CBD, which was recently approved by the FDA as an adjunct to conventional ASDs, significantly reduces seizure frequency in patients.[13,30] In the clinic, CBD is used as add-on therapy to currently prescribed treatment, with 66% of patients using it concomitantly with CLB.[30] In this present study we tested CBD as a monotherapy and found that 200 mg/kg CBD did not offer significant protection against hyperthermia-induced seizures (Table 2). Although there was no protective effect in this model at the dose and time point tested, CBD monotherapy showed significant protection in two other DS mouse models. When 100 mg/kg CBD was administered to the *F1.Scn1a*^{tm1Kea} mouse it significantly increased the temperature at which mice seized[27] and when administered to the *Scn1a*$^{+/-}$ null mouse at both 100 mg/kg and 200 mg/kg, CBD significantly reduced seizure length and severity.[35] In the *F1.Scn1a*^{tm1Kea} mouse, the combination of 10 mg/kg CLB and 12 mg/kg CBD significantly increased the temperature threshold as compared to CBD monotherapy, suggesting that the combination therapy may be more

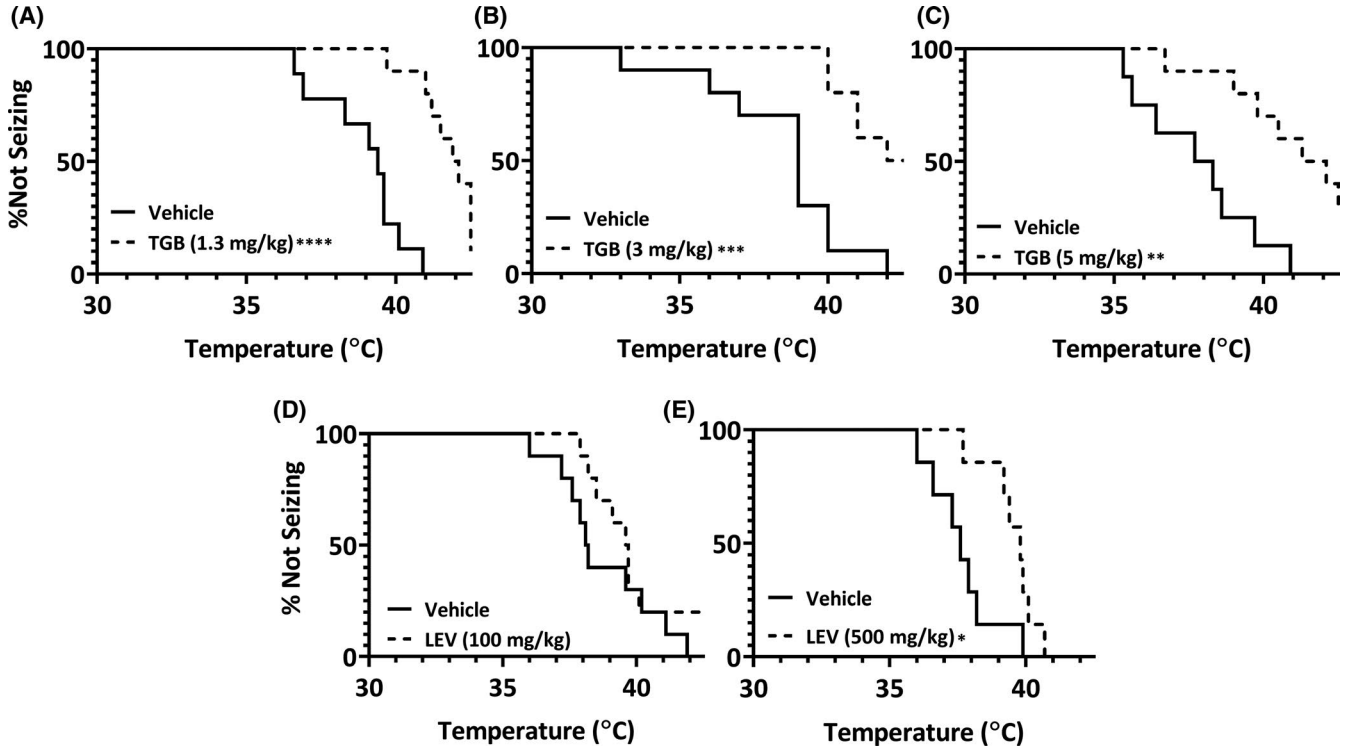

FIGURE 3 Evaluation of tiagabine (TGB) and levetiracetam (LEV). TGB and LEV were evaluated at different doses to determine the temperature thresholds for hyperthermia-induced seizures. (A) A dose of 1.3 mg/kg (TGB) significantly increased the temperature threshold at which mice seized as compared to vehicle ($p < .0001$) with 1 of 10 mice completely protected against hyperthermia-induced seizures (vehicle: $n = 9$; TGB (1.3 mg/kg): $n = 10$). (B) A dose of 3 mg/kg (TGB) significantly increased the temperature at which mice had hyperthermia-induced seizures as compared to vehicle ($p = .0003$), with 3 of 9 mice demonstrating complete protection (vehicle: $n = 10$; TGB (3 mg/kg): $n = 9$). (C) A dose of 5 mg/kg (TGB) significantly increased the temperature at which mice had seizures ($p = .0014$) with 3 of 10 showing complete protection (vehicle: $n = 9$; TGB (5 mg/kg): $n = 10$). (D) LEV administered at a dose 100 mg/kg did not confer significant protection against hyperthermia-induced seizures ($p = .2242$). However, two mice were completely protected from seizure activity (vehicle: $n = 10$, LEV (100 mg/kg): $n = 10$). (E) Increasing the dose of LEV to 500 mg/kg conferred significant protection against hyperthermia-induced seizures ($p = .0253$) (vehicle: $n = 7$, LEV (500 mg/kg): $n = 7$), $*p < .05$ $**p < .01$, $***p < .001$, $****p < .0001$; Log-rank (Mantel-Cox)

beneficial. In addition, because not all people with DS have their seizures suppressed by CBD, not all mouse models of DS are protected from hyperthermia-induced seizures.

When DS is treated, sodium channel blockers, such as CBZ and LTG, are avoided, as these can make seizure frequency and severity worse.[9,36] In *Scn1a*$^{A1783V/WT}$ mice, a single injection of 40 mg/kg CBZ significantly lowered the temperature at which mice seized (Figure 2). Although a single dose of 10 mg/kg LTG had no effect on threshold temperatures (Figure S3, Table 2), sub-chronic administration of 20 mg/kg LTG over the course of 5 days significantly lowered the temperatures at which mice seized and two mice died due to treatment (Figure S3D, Table 2). Both CBZ and LTG were tested in the *F1.Scn1a*^{tm1Kea} model, but a single dose of 20 mg/kg LTG significantly lowered temperature threshold, whereas 20 mg/kg CBZ had no effect. Our study did not include single administration of 20 mg/kg LTG; however, we presume it would also lower the temperature threshold, much like observed in the *F1.Scn1a*^{tm1Kea}. The CBZ doses differed between the two studies, which could contribute to the difference seen in the temperature thresholds. However, given that our results align with what is seen in the clinical setting, these compounds functioned as hypothesized in the *Scn1a*$^{A1783V/WT}$ mouse model.

A notable effect in the present study was that TGB conferred protection against hyperthermia-induced seizures in *Scn1a*$^{A1783V/WT}$ (Figure 3). This is consistent with the pharmacological profile in a haploinsufficiency mouse model, as a high dose of TGB (10 mg/kg) significantly protected mice against generalized tonic-clonic seizures during hyperthermia testing.[31] Although we saw significant protection in our model, with minimal toxic side effects, in patients, TGB is associated with behavioral side effects and reports of non-convulsive absence and myoclonic seizures associated with TGB treatment.[37] Therefore, NICE guidelines recommend against using TGB for treatment of DS. In addition to TGB, in the present study, LEV, at a dose of 500 mg/kg, significantly reduced the temperature at which mice seized (Figure 3E). LEV also showed efficacy in the *F1.Scn1a*^{tm1Kea} mouse model, albeit at a dose much lower (10 mg/kg) than used

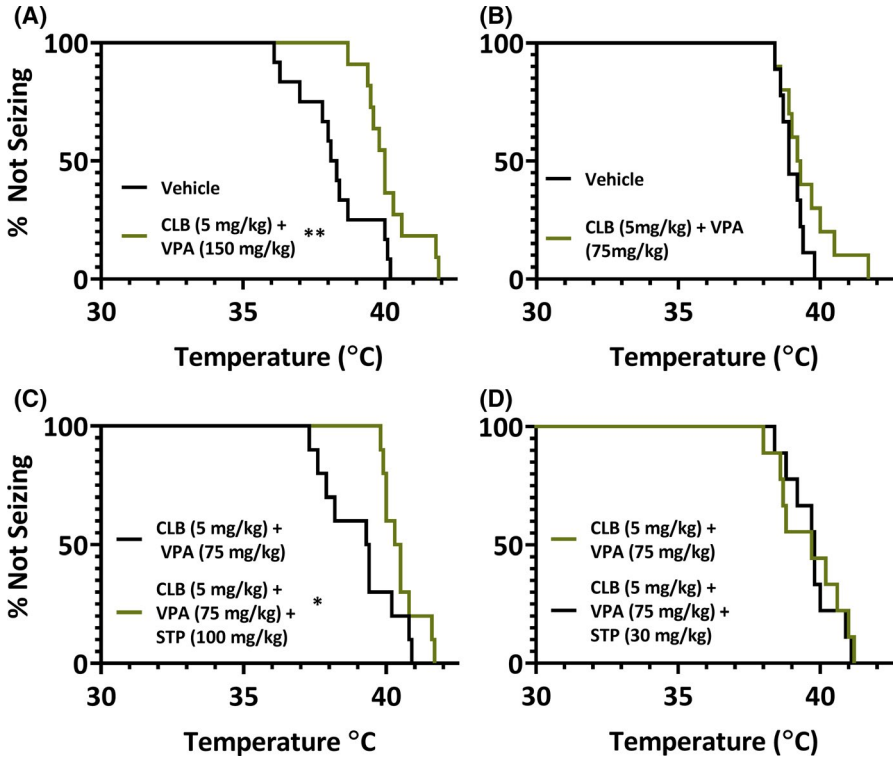

FIGURE 4 Add-on therapy for the treatment of Dravet syndrome. (A) Clobazam (CLB) [5 mg/kg] and valproic acid (VPA) [150 mg/kg] treatment significantly lowered the temperature threshold of hyperthermia-induced seizures as compared to vehicle-treated mice (vehicle: $n = 12$, CLB + VPA: $n = 11$, $p = .0060$). (B) CLB (5 mg/kg) and VPA (75 mg/kg) did not significantly lower the temperature at which *Scn1a*$^{A1783V/WT}$ mice had hyperthermia-induced seizures as compared to vehicle-treated mice (vehicle: $n = 9$, CLB + VPA: $n = 10$, $p = .1511$). (C) The three drug combo of CLB (5 mg/kg), VPA (75 mg/kg) and stiripentol (STP) [100 mg/kg] offered significant protection against hyperthermia-induced seizures as compared to CLB (5 mg/kg) + VPA (75 mg/kg) treated *Scn1a*$^{A1783V/WT}$ mice (CLB + VPA + STP: $n = 10$, CLB + VPA: $n = 10$, $p = .0471$). (D) CLB (5 mg/kg) + VPA (75 mg/kg) + STP (30 mg/kg) did not significantly the temperature at which mice seized as compared to CLB (5 mg/kg) + VPA (75 mg/kg) treated mice (CLB + VPA + STP: $n = 9$, CLB + VPA: $n = 9$ $p = .7973$) *$p < .05$, **$p < .01$; Log-rank (Mantel-Cox)

here.[18] LEV is used when no other first-line treatment is successful,[32] with responder rates anywhere between 11% and 64%[38,39] in individuals with DS. This may explain the variability reported between mouse models.

Although CLB is commonly used in the treatment of DS, its responder rate is ~28%[32] and add-on treatments are normally required to obtain adequate seizure control. These include the addition of drugs such as VPA and STP. VPA monotherapy (300 mg/kg) did not offer significant protection as compared to vehicle-treated mice in the present study (Table 2). Thus we combined 5 mg/kg CLB and 150 mg/kg VPA and found that it conferred significant protection (Figure 4A). We decreased the dose of VPA to 75 mg/kg, while maintaining CLB at a dose of 5 mg/kg and found it no longer significantly protected *Scn1a*$^{A1783V/WT}$ mice (Figure 4B). When the combination of CLB and VPA fails in the clinic, STP is added to the treatment regimen.[11] Therefore, we administered 5 mg/kg CLB, 75 mg/kg VPA, and 100 mg/kg STP and found that this offered significant protection as compared to 5 mg/kg CLB and 75 mg/kg VPA treatment (Figure 4C). We found this triple-drug therapy to be effective and the results align with the use of this add-on pharmacological treatment regimen for patients with DS. The combined use of CLB and VPA with an add-on investigational compound may be a useful approach toward novel therapy screening in this mouse model of DS.

Additional compounds were tested against hyperthermia-induced seizures in the *Scn1a*$^{A1783V/WT}$ mouse model. Much like what is observed in the clinical setting with patients, this model is highly pharmacoresistant. However, because most of these experiments were all performed with acute injections, it might prove useful to determine the effect of these compounds when sub-chronically administered at doses sufficient to provide steady-state therapeutic brain and plasma levels. Nevertheless, those experiments are technically challenging given the stress of multiple injections per day over the course of several days. Therefore, subchronic dosing, wherein drugs can be delivered in food, would be a potential approach to addressing the limitations inherent in performing subchronic dosing experiments with injections.

Another phenotype of DS is spontaneous, recurrent seizures. Drugs effective in patients, such as treatment with

CBD (Table 2) and FFA (Figure S3 A,B, Table 2), may be effective at reducing spontaneous seizure frequency in this mouse model or require chronic dosing in hyperthermia studies. In studies where conventional ASDs were screened in other mouse models of DS, variable results between hyperthermia-induced and spontaneous seizures were achieved. In the *F1.Scn1a*^{tm1Kea} mouse model, CLB was protective against hyperthermia-induced seizures but had minimal effect against spontaneous seizures.[18] Surprisingly, in the *F1.Scn1a*^{tm1Kea} mouse, CBZ had no effect on either hyperthermia-induced or spontaneous seizures, whereas we saw a significant effect in lowering the temperature threshold of hyperthermia-induced seizures.[18] In another study using the *F1.Scn1a*^{tm1Kea} mouse, monotherapy CBD significantly increased the temperature threshold of mice, yet had no effect on spontaneous seizure frequency.[27] Therefore, it is possible that there is a pharmacological difference between hyperthermia-induced and spontaneous seizures, which may explain a lack of translation of some compounds to the clinic. In order to find effective therapies, novel therapeutics may also need to be screened against spontaneous seizures. Nonetheless, using hyperthermia-induced seizures is still an important screening platform as due to its highly pharmacoresistant nature we may be able to uncover extremely effective drugs.

Beyond the differences that may exist between hyperthermia-induced and spontaneous seizures at a pharmacological level, there are more than 900 distinct mutations that result in DS,[20] making this form of epilepsy highly patient specific. Consequently, it is possible that mice will respond differently to ASDs when exhibiting different mutations. To determine why differences exist between models expressing different mutations, a large study comparing pharmacokinetic (pk) data for each drug tested in each model would be needed. It is possible that innate differences in febrile seizures and drug exposure may exist for knock-out and missense mutations. This could explain why some of the ASDs have shown to be effective against hyperthermia-induced seizures in the present model, which results from a missense mutation, and not in the *F1.Scn1a*^{tm1Kea} mouse, a model of haploinsufficiency. Therefore, we not only need translatable models for hyperthermia-induced and spontaneous seizures, but it would also be beneficial to have well-characterized models for the different mutations, including pk and exposure data for the drugs tested.

An additional approach to screening novel compounds for DS is the use of zebrafish larvae. Although zebrafish larvae do not recapitulate all the aspects of DS, such as cognitive disabilities, the *scn1a*^{s552} larvae exhibit unprovoked seizures.[40] This particular model has been validated with a number of ASDs, and the suppression of unprovoked seizure activity was achieved with commonly prescribed drugs for patients with DS,[41] whereas drugs such as sodium channel blockers increased seizure activity. This model is particularly advantageous, as high throughput with short assay times is achieved, allowing rapid screening of novel compounds, a significant advantage over mouse models. In fact, lorcaserin, which has shown efficacy in some patients with DS, was identified in zebrafish larvae,[42] further demonstrating that this model has a role in the discovery of treatments for DS.

Overall, the present study sets a foundation for screening novel compounds developed for DS using the *Scn1a*$^{A1783V/WT}$ model. Although the *Scn1a*$^{A1783V/WT}$ mouse did not respond to all drugs used as treatments for DS, the hyperthermia-induced seizure phenotype was highly pharmacoresistant, suggesting that this model could lead to the discovery of novel and robust compounds for the treatment of DS, especially when paired with CLB and VPA treatment, as is done clinically.

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CONFLICT OF INTEREST

None of the authors has any conflict 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.

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