ANNALS of Clinical and Translational Neurology
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AMERICAN NEUROLOGICAL ASSOCIATION

BRIEF COMMUNICATION

Depressed glutamate transporter 1 expression in a mouse model of Dravet syndrome

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Received: 11 January 2023; Revised: 6 June 2023; Accepted: 27 June 2023

***Annals of Clinical and Translational Neurology* 2023; 10(9): 1695–1699**

doi: 10.1002/acn3.51851

Abstract

Dravet syndrome (DS) is a monogenic, often refractory, epilepsy resultant from *SCN1A* haploinsufficiency in humans. A novel therapeutic target in DS that can be engaged in isolation or as adjunctive therapy is highly desirable. Here, we demonstrate reduced expression of the rodent glutamate transporter type 1 (GLT-1) in a DS mouse model, and in wild type mouse strains where *Scn1a* haploinsufficiency is most likely to cause epilepsy, indicating that GLT-1 depression may play a role in DS seizures. As GLT-1 can be upregulated by common and safe FDA-approved medications, this strategy may be an attractive, viable, and novel avenue for DS treatment.

Introduction

Heterozygous loss-of-function mutations in the human *SCN1A* gene, which encodes the alpha subunit of the heteromeric neuronal voltage-gated sodium channel $Na_V1.1$, result in Dravet syndrome (DS), a severe infantile-onset monogenic epileptic encephalopathy characterized by intractable seizures, developmental delays, and increased mortality.[1] The DS phenotype expresses variably among individuals with the same mutation, suggesting that genetic or environmental modifiers influence disease severity. The need for novel anticonvulsants in DS is underscored by recent FDA clearance of three compounds in the DS space, and active research into gene therapies.[2–4] Unfortunately, none of the newly approved drugs fully suppress seizures in DS,[2] and there is no indication yet that any gene therapy will lead to seizure freedom in treated patients.[4]

We and others have identified impaired synaptic glutamate clearance due to reduced expression of glutamate transporter type 1 (GLT-1) as a contributor to epileptogenesis.[5–8] GLT-1 (termed excitatory amino acid transporter type 2, EAAT2, in humans) is highly expressed, and is the major determinant of glutamate clearance from excitatory synapses.[5,9] Given its critical role in glutamate homeostasis, GLT-1/EAAT2 reduction logically corresponds to excess excitatory signaling and seizures, and to excitotoxic injury of vulnerable inhibitory interneuron populations, which are already compromised by the *SCN1A* haploinsufficiency in DS. Notably, GLT-1 expression is a druggable target as it can be enhanced by a number of safe and inexpensive compounds, including common β lactam antibiotics,[6,10] which raises prospects for these molecules to contribute to seizure control in DS.

Here, we test whether cortical GLT-1 protein expression is depressed in *Scn1a* haploinsufficient mouse DS models on both the 129S1/SvImJ and an F1 hybrid (C57BL/6Jx129S1/SvImJ) background[11] as a step toward testing GLT-1 upregulation as a plausible novel therapeutic strategy in DS. Interestingly, on the 129S background, heterozygous $Scn1a^{+/-}$ mice do not exhibit seizures and have a normal lifespan,[11] while $Scn1a^{+/-}$ mice on the C57 background develop spontaneous seizures and have a high mortality.[12] When these two strains are crossed, heterozygotes on an F1 hybrid (C57x129S) background exhibit a less severe epileptic phenotype and an intermediate mortality.[11]

Given the apparent contribution of modifier alleles that unmask $Scn1a^{+/-}$ seizure susceptibility by the C57

strain,[11,13] we also measured baseline GLT-1 in wild type (WT) mice of all three strains.

Materials and Methods

Animals

All experiments were approved by the Institutional Animal Care and Use Committee at Boston Children's Hospital and in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals. 129S-*Scn1a*^{tm1Kea}/Mmjax males (MMRRC stock #037107-JAX)[11] were bred to wild type 129S1/SvImJ (129S WT) females (JAX stock #002448) to maintain a live colony of 129S *Scn1a*$^{+/-}$ heterozygotes (129S HET). 129S HET males were then crossed with wild type C57BL/6J (C57 WT) female mice (JAX stock #000664) to generate F1 hybrid (C57x129S) wild type (F1 WT) and *Scn1a*$^{+/-}$ heterozygous (F1 HET) animals. All experiments were performed in mixed-sex, age-matched cohorts.

Video electroencephalography (video EEG)

Adult P83 mice underwent wireless telemetry transmitter (ETA-F10; DSI, MN) implantation with skull screw electrodes (active: right parietal cortex; reference: left olfactory bulb) as described previously.[14,15] After 1 week of postoperative recovery (i.e., at P90), 1 week of continuous epidural one-channel EEG was recorded from each mouse, sampled at 1 kHz and coupled with continuous video (Ponemah Software v6.51; DSI, MN), as described previously.[14] All recordings were by wireless telemetry in each mouse's home cage. EEG data were scored for generalized tonic–clonic (GTC) seizures using a semiautomated seizure detection algorithm (Neuroscore 3.4.1; DSI, MN) where automatically marked events were verified by visual review of real-time video and spectrogram.[15] Seizure frequency and mean seizure duration were calculated per mouse.

Cortical protein extraction

P90 mice were decapitated, and neocortex was dissected over ice. Tissue was flash-frozen in liquid nitrogen and stored at −80°C until use for protein analysis. Tissue was Dounce homogenized (25 strokes) in ice-cold homogenization buffer (20 mL/g tissue mass) containing 1% SDS in 50 mM phosphate buffer (pH 7.4) and one tablet each of cOmplete^{TM}, Mini protease inhibitor and PhosSTOP^{TM} phosphatase inhibitor cocktails per 10 mL of buffer (Roche, Germany). Lysates were vortexed for 15 s before centrifugation at 13,000 rpm at 4°C.

Western blot

Samples for SDS-PAGE were prepared in 4× Laemmli Sample Buffer (Bio-Rad, CA), and separated on 4%–20% gradient gels (Criterion TGX, Bio-Rad, CA). After transfer to PVDF membranes, immunoblotting was performed using specific primary antibodies for GLT-1 protein (cat. # 250 203; Synaptic Systems GmbH, Germany), with β-actin as a within-sample loading control (cat. # 251 011; Synaptic Systems GmbH, Germany). Imaging was conducted using a near-infrared western blot detection system (Odyssey® CLx, LI-COR Biosciences, NE). In accordance with long-standing laboratory protocol, single lanes with incomplete protein bands due to bubble artifacts and lanes with—and neighboring—conspicuously smeared bands were excluded from analysis. GLT-1 was normalized to β-actin per sample then averaged across technical and biological replicates on repeat western blots.

Statistical analysis

Data were compared across experimental groups using Fisher's exact, Mann–Whitney U, and unpaired *t*-tests as needed, with the significance level set at $p < 0.05$. Results are presented as median or mean ± SEM.

Results

Scn1a haploinsufficiency results in GTC seizures, in some, but not all F1 mice

Consistent with published reports,[11] video EEG identified GTC seizures in 65.7% of F1 HET mice, and none in F1 WT mice (Fisher's exact $p = 0.002$) (Fig. 1A). For the entire recorded F1 HET population, medians for seizure frequency and mean seizure duration per mouse corresponded to 4.0 GTC seizures/week (Mann–Whitney U test; $p = 0.004$; Fig. 1B), and 37.2 s (Mann–Whitney U test; $p = 0.004$; Fig. 1C), respectively. Mean seizure frequency in F1 HET mice with GTC seizures was 5.7 ± 0.5 per week and mean seizure duration was 45.1 ± 2.2 s.

Cortical GLT-1 is depressed in both F1 (C57x129S) and 129S *Scn1a*$^{+/-}$ mutant mice

GLT-1 protein expression was reduced in F1 HET mice compared to F1 WT littermates (F1 WT: 100.0 ± 2.2%; F1 HET: 92.0 ± 2.0%; unpaired *t*-test $p = 0.017$) (Fig. 2A,B), as well as in 129S HET animals compared to

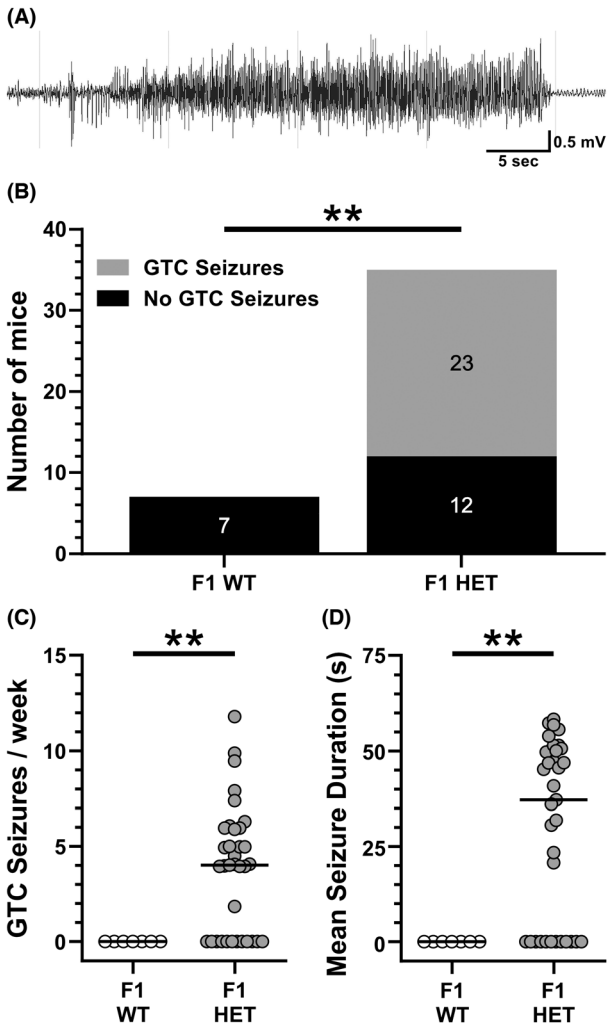

Figure 1. Seizure activity in F1 HET mice. (A) Representative EEG trace showing spontaneous GTC seizure activity in an F1 HET mouse. (B) Compared to F1 WT littermates, F1 HET mice exhibit GTC seizure activity (Fisher's exact test; $^{**}p < 0.01$). (C, D) Per-animal, convulsive seizure frequency and mean seizure duration are significantly higher in F1 HET compared to F1 WT controls. Bars indicate median (Mann–Whitney U test; $^{**}p < 0.01$).

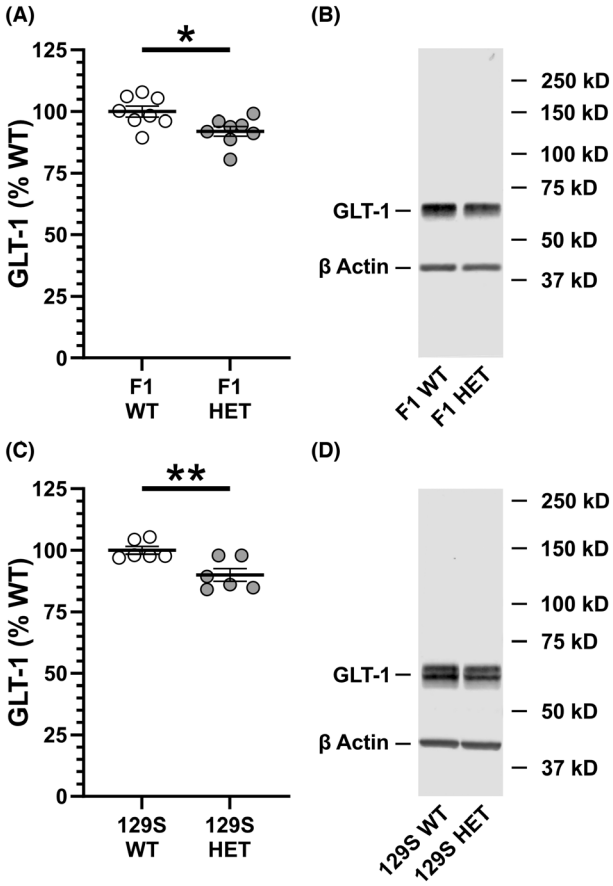

Figure 2. Cortical GLT-1 expression in F1 and 129S *Scn1a*$^{+/-}$ mice. (A) F1 HET mice have decreased cortical GLT-1 expression compared to F1 WT littermates. (B) Representative immunoblot of cortical GLT-1 expression in F1 WT and F1 HET groups. (C) Cortical GLT-1 expression is also lower in 129S HET mice than in 129S WT littermate controls. (D) Representative immunoblot of cortical GLT-1 expression in 129S WT and 129S HET mice. Bars indicate mean ± SEM (unpaired *t*-test; $^{*}p < 0.05$, $^{**}p < 0.01$).

controls (129S WT: 100.0 ± 1.6%; 129S HET: 90.0 ± 2.6%; unpaired *t*-test $p = 0.008$) (Fig. 2C,D).

Cortical GLT-1 expression in wild type mice is strain-dependent

Background strain-specific differences in GLT-1 protein expression were also observed, with adult 129S WT mice exhibiting higher cortical GLT-1 expression compared to both F1 WT (129S WT: 100.0 ± 3.4%; F1 WT: 80.4 ± 3.8%; unpaired *t*-test $p = 0.003$) (Fig. 3A,B) and C57 WT mice (129S WT: 100.0 ± 3.0%; C57 WT: 83.6 ± 4.5%; unpaired *t*-test $p = 0.014$) (Fig. 3C,D).

Discussion

While GLT-1 biology has been studied extensively in wild type mice,[16] and in some rodent epilepsy models,[6,10,17,18] GLT-1 expression and function patterns in DS are largely unknown. We demonstrate for the first time that cortical GLT-1 expression is reduced in a *Scn1a* haploinsufficient mouse DS model and identify that GLT-1 protein expression also varies between mouse background strains. Specifically relevant to interstrain GLT-1 differences, we note that the two strains where seizures with *Scn1a* haploinsufficiency are likely (C57 and F1 C57x129S), have lower GLT-1 expression than the 129S strain which appears seizure-resistant in the *Scn1a* haploinsufficiency setting.[11,12] These results indicate that (1) GLT-1/EAAT2- may be depressed in DS, and (2) variable background

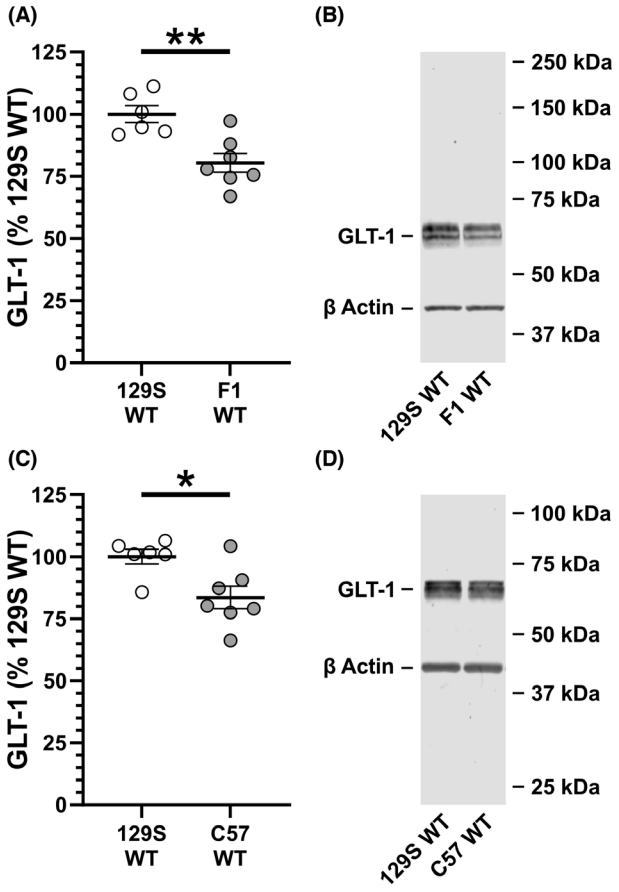

Figure 3. Strain differences in GLT-1 expression among wild type mice. (A) Cortical GLT-1 expression is decreased in F1 WT mice compared to 129S WT controls. (B) Representative immunoblot of cortical GLT-1 expression in 129S WT and F1 WT animals. (C) C57 WT also exhibit lower cortical GLT-1 protein expression than 129S WT controls. (D) Representative immunoblot of cortical GLT-1 expression in 129S WT and C57 WT mice. Bars indicate mean ± SEM (unpaired *t*-test; $*p < 0.05$, $**p < 0.01$).

GLT-1/EAAT2 background expression may contribute to the clinical severity of *SCN1A* mutations, thus raising prospects for improving seizure control in patients with DS by GLT-1/EAAT2 upregulation.

Our results point to a conundrum, answering which is essential for understanding GLT-1 biology in DS and is also more broadly relevant to GLT-1-mediated glutamate homeostasis in epilepsy. Specifically, does reduced GLT-1 expression lead to recurrent seizures, or do recurrent seizures depress GLT-1 expression? Published reports and our prior work support both scenarios: cortical GLT-1 is reduced by epileptogenic traumatic brain injury[6,10] and by pentylenetetrazole (PTZ)-induced seizure kindling,[19] and hippocampal *Slc1a2* mRNA is decreased in young seizing F1 *Scn1a*$^{+/-}$ mice.[20] Conversely, genetic GLT-1 loss leads to fatal epilepsy in knockout mice,[5] and to an epileptic encephalopathy in humans.[21]

Our findings argue against seizure-driven GLT-1 reduction in *Scn1a*$^{+/-}$ mutant mice. *Scn1a* haploinsufficiency on the 129S background has been reported to *not* result in an overt epileptic phenotype.[11,12] In contrast, in F1 hybrid (C57x129S) *Scn1a*$^{+/-}$ and C57 *Scn1a*$^{+/-}$ mice, the haploinsufficiency leads to intermediate and severe epileptic phenotypes, respectively.[11,12] Several Dravet syndrome modifier (*Dsm*) loci have been identified in *Scn1a* haploinsufficient mice that may be responsible for this strain-dependent difference.[11,13] However, cortical GLT-1 (or its gene, *Slc1a2*) contribution to such seizure susceptibility has not been described previously. Yet we see almost identical reductions in GLT-1 expression in both F1 and 129S *Scn1a*$^{+/-}$ mice, indicating that reduced GLT-1 expression may be an endophenotype of *Scn1a* haploinsufficiency itself. Our results thus suggest that a second GLT-1 "hit" in a susceptible background (i.e., a genotype that corresponds to reduced wild type GLT-1 expression and other unfavorable modifier alleles) by way of an *Scn1a* mutation may shift mice (or patients) over a threshold into an epileptic phenotype.

Future experiments and therapeutic trials will aim to answer this important question of causality, and whether GLT-1 upregulation would work best to prevent seizure onset or mitigate established seizures. Previous preclinical studies have demonstrated that successful and sustained endogenous GLT-1 upregulation renders rats resistant to PTZ kindling,[19] and that *ex post facto* upregulation via genetic or pharmacological strategies alleviates established seizures,[22] exemplifying the potential of such an approach in DS.

In summary, our findings indicate that GLT-1/EAAT2 expression may be a viable therapeutic target in DS and underscore that modulation of background neurobiology elements can be considered in complement to treating the primary deficit in the genetic epilepsies.

Author Contributions

Mustafa Q. Hameed and Alexander Rotenberg contributed to conception and experimental design. All authors contributed to acquisition and analysis of data, drafting the text, and/or preparation of figures.

Acknowledgments

We thank the IDDRC Animal Behavior and Physiology Core at Boston Children's Hospital, funded by NIH/NICHD P50 HD105351. This work was supported by NIH/NINDS 1R21NS123459 (AR) and the Boston Children's Hospital Translational Research Program (AR).

Conflict of Interest Statement

The authors have no conflict of interest to declare.

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