Fine mapping and candidate gene analysis of a dravet syndrome modifier locus on mouse chromosome 11

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Abstract
Pathogenic variants in SCN1A result in a spectrum of phenotypes ranging from mild febrile seizures to Dravet syndrome, a severe infant-onset epileptic encephalopathy. Individuals with Dravet syndrome have developmental delays, elevated risk for sudden unexpected death in epilepsy (SUDEP), and have multiple seizure types that are often refractory to treatment. Although most Dravet syndrome variants arise de novo, there are cases where an SCN1A variant was inherited from mildly affected parents, as well as some individuals with de novo loss-of-function or truncation mutations that presented with milder phenotypes. This suggests that disease severity is influenced by other factors that modify expressivity of the primary mutation, which likely includes genetic modifiers. Consistent with this, the Scn1a+/− mouse model of Dravet syndrome exhibits strain-dependent variable phenotype severity. Scn1a+/− mice on the 129S6/SvEvTac (129) strain have no overt phenotype and a normal lifespan, while [C57BL/6Jx129]F1.Scn1a+/− mice have severe epilepsy with high rates of premature death. Low resolution genetic mapping identified several Dravet syndrome modifier (Dsm) loci responsible for the strain-dependent difference in survival of Scn1a+/− mice. To confirm the Dsm5 locus and refine its position, we generated interval-specific congenic strains carrying 129-derived chromosome 11 alleles on the C57BL/6J strain and localized Dsm5 to a 5.9 Mb minimal region. We then performed candidate gene analysis in the modifier region. Consideration of brain-expressed genes with expression or coding sequence differences between strains along with gene function suggested numerous strong candidates, including several protein coding genes and two miRNAs that may regulate Scn1a transcript.

Introduction

Dravet syndrome is an infant-onset epileptic encephalopathy caused by haploinsufficiency for SCN1A. Seizure onset usually occurs between 6 and 18 months of age with generalized tonic–clonic or hemiclonic seizures, often precipitated by fever. Individuals subsequently develop pleomorphic afebrile seizure types that often respond poorly to conventional therapies (Dravet 2011; Dravet and Oguni 2013; Wirrell et al. 2017). Development is normal prior to onset, but disease progression is frequently accompanied by stagnation or decline of psychomotor and cognitive development (Battaglia et al. 2021; Dravet 2011). Individuals with Dravet syndrome have a significantly increased risk of mortality, mainly attributed to prolonged status epilepticus in early childhood and sudden unexplained death in epilepsy (SUDEP) in adolescents and adults (Dravet 2011). In most cases, the SCN1A variants arise de novo and result in heterozygous loss-of-function (Li et al. 2021). However, there have been reports of Dravet syndrome caused by SCN1A variants inherited from mildly affected parents, as well as some individuals with de novo loss-of-function or premature truncation variants that presented with milder phenotypes, like generalized epilepsy with febrile seizures plus (GEFS+) (Depienne et al. 2010, 2009; Goldberg-Stern et al. 2014; Guerrini et al. 2010; Nabbout et al. 2003; Osaka et al. 2007; Yordanova et al. 2011; Yu et al. 2010). This variable expressivity suggests that disease severity is influenced by additional factors, which may include genetic modifiers (de Lange et al. 2020; Hammer et al. 2017).
Mice with heterozygous targeted deletion of Scn1a (Scn1a<sup>+</sup><sup>−</sup>) are a well-validated model and recapitulate core features of Dravet syndrome, including epilepsy and sudden unexpected death following a seizure in otherwise healthy animals (SUDEP-like) (Kalume et al. 2013; Miller et al. 2014; Yu et al. 2006). The highest incidence of SUDEP-like deaths occurs in the 4th postnatal week and survival stabilizes after 6 weeks of age (Kalume et al. 2013; Miller et al. 2014). Genetic background dramatically influences phenotype severity and survival of Scn1a<sup>−/−</sup> mice (Miller et al. 2014; Rubinstein et al. 2015; Yu et al. 2006). On the 129S6/SvEvTac (129) strain, there is no overt phenotype and 129.Scn1a<sup>−/−</sup> mice live a normal lifespan. However, when 129.Scn1a<sup>−/−</sup> mice are crossed with C57BL/6J (B6), the resulting [129xB6]F1.Scn1a<sup>−/−</sup> mice (F1.Scn1a<sup>−/−</sup>) have a severe phenotype with spontaneous seizures and premature lethality (Miller et al. 2014). We previously performed genetic mapping and identified several Dravet syndrome modifier (Dsm) loci that influence the strain-dependent difference in survival (Miller et al. 2014).

In this study, we used interval-specific congenic (ISC) strains to fine map the Dsm5 locus on chromosome 11 and identified a minimal interval that influenced survival, particularly in female Scn1a<sup>−/−</sup> mice. Genotyping of microsatellite markers was performed by analysis of PCR products on 7% denaturing polyacrylamide gels stained with ethidium bromide. ISC breakpoints were refined using the mini Mouse Universal Genotyping Array (miniMUGA) (Neogen, Lincoln, NE, USA). Strain background was surveyed for allele specific primers (WT: 5'-CCC TGA GAT GTG GGT AGA CTG CCT TGG GAA AAG CG; KO: 5'-AGT CTG TAC CAG GCA GAA CTTG) and two multiplex PCR using a common primer (5'-AGTCTGTACCGCCAGAAGTGCGA TAG; KO: 5'-AGACTGCTTTGGGAAAGCGC). Amplicons include a 357 bp WT product and a 200 bp KO product. Genotyping was performed by analysis of PCR products on 7% denaturing polyacrylamide gels stained with ethidium bromide. ISC lines were crossed to B6 for ≥ N9 generations prior to any experiments.

**Methods**

**Mice (NCBI Taxon ID 10090)**

Scn1a<sup>tm1Kea</sup> mice (MMRRC 037107-JAX) were generated by homologous recombination in TL1 ES cells (129S6/SvEvTac) as previously described (Miller et al. 2014). The line has been maintained as isogenic on 129S6/SvEvTac (129) by continuous backcrossing of 129.Scn1a<sup>−/−</sup> to 129 inbred mice (129SVE, Taconic Biosciences, Germantown, NY, USA). Trpv1 knockout mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA; RRID:IMSR_JAX:003770). C57BL/6J mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA; RRID:IMSR_JAX:000664).

Mice were maintained in a Specific Pathogen Free (SPF) barrier facility with a 14:10 light:dark cycle and ad libitum access to food and water. 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 were considered when planning experiments (Percie du Sert et al. 2020).

**Interval specific congenic (ISC) lines**

We generated nine ISC lines carrying 129-derived alleles on chromosome 11 on a C57BL/6 J (B6) background, designated as B6.129-Dsm5A through B6.129-Dsm5F. F1 progeny were generated by crossing 129 males with B6 females, and then successively crossed to B6 to generate congenic lines. Genotyping for chromosome 11 markers performed at each generation and mice retaining 129 alleles were propagated. Whole genome and selective genotyping was performed at generations N2 and N5 to select breeders with low percentages of 129 in the rest of the genome. All lines were crossed to B6 for ≥ N9 generations prior to any experiments.

**Genotyping**

DNA was prepared from tail biopsies (Gentra Puregene Mouse Tail Kit, Qiagen, Valencia, CA, USA). Scn1a genotype was determined by multiplex PCR using a common primer (5'-AGTCTGTACCGCCAGAACTTG) and two allele specific primers (WT: 5'-CCCTGAGATGTGGTGAATAG; KO: 5'-AGACTGCTTTGGGAAAGCG). Amplicons include a 357 bp WT product and a 200 bp KO product. Genotypes of microsatellite markers was performed by analysis of PCR products on 7% denaturing polyacrylamide gels stained with ethidium bromide. ISC lines were crossed to B6 for ≥ N9 generations prior to any experiments.

**Phenotyping**

B6.129-Dsm5 females were bred with heterozygous 129.Scn1a<sup>−/−</sup> males to generate F1 offspring carrying homozygous 129/129 alleles or heterozygous 129/B6 alleles in Dsm5. Offspring were ear-tagged and genotyped at P12-14. At P19-21, mice were weaned into standard vivarium cages containing 4–5 mice of the same sex and age. Wild-type littermates were included in holding cages. Survival was monitored to 8 weeks of age. Over the 8-week period, mice were monitored daily for general health and any visibly unhealthy mouse (e.g., underweight, dehydrated, poorly groomed, or immobile) was euthanized and excluded from the study; this occurred rarely. The focus of the study was sudden and unexpected death in otherwise healthy appearing Scn1a<sup>−/−</sup> mice. Survival was compared between groups using Kaplan–Meier analysis with p-values determined by
LogRank Mantel–Cox tests. Group sizes were based on data simulations using data from our prior survival studies.

**Candidate gene analysis**

We defined the *Dsm5* gene set using the Mouse GRCm38.p6 reference genome (B6) in Ensembl BioMart, which included classification by gene type. To define a subset of genes with CNS expression, we used the MGI database and EMBL–EBI Expression Atlas. Differential expression of *Dsm5* genes between 129 and B6 or F1 was assessed using two RNA-Seq datasets that we previously reported: (1) B6 and 129 forebrain (Hawkins et al. 2016); and (2) F1 and 129 hippocampus (Hawkins et al. 2019) (NCBI GEO GSE112627). Differential expression of miRNAs was assessed using a previously reported dataset (Trontti et al. 2018). To identify consequential coding sequence changes between the strains, we performed whole genome re-sequencing of 129S6/SvEvTac and compared with the C57BL/6J reference sequence and mouse strains in Ensembl (Mouse GRCm38. p6) (NCBI SRA PRJNA817075). The effect of single-nucleotide variants (SNVs) was assessed using the Ensembl Variant Effect Predictor tool (VEP) (McLaren et al. 2016), and SNVs classified as deleterious were retained. TargetScan and LncRRIsearch were used to predict targets of *Dsm5* high confidence miRNAs and lncRNAs, respectively (Agarwal et al. 2015; Fukunaga et al. 2019).

**Results**

**Fine mapping of *Dsm5* to central chromosome 11**

Low resolution mapping of *Dsm5* on chromosome 11 localized a 1.5-LOD support interval to 4.7–39.7 cM (6.99–63.9 Mb; GRCm38.p6) (Miller et al. 2014). To refine the map interval, we used ISC lines carrying varying 129-derived chromosome 11 segments on a congenic B6 background (Fig. 1A; Table 1). Each B6.129-Scn1a strain was crossed to 129, and F1 mice in pooled samples containing both sexes (Fig. 2A; B); and three DEGs between P14 or P24 wild-type 129 and F1 mice in pooled samples containing both sexes (Pafah1b1, Ywhae, 6330403E22Rik) (Fig. 2C-E). There were three DEGs (*Rtn4rl1, Serpinf1, Smyd4*) when comparing F1-*Scn1a*+/+ mice without seizures and F1-*Scn1a*−/− mice with recent seizures (Fig. 2F-H). From whole genome sequencing, we identified 24 genes in the refined *Dsm5* interval with predicted deleterious variants, including missense, splice site, and indels (4930563E22Rik, 4933427D14Rik, 6330403K07Rik, Aspa, Cluh, E130309D14Rik, Pimreg, Haspin, Hic1, Itgae, Nlrp1a, Nlrp1b, Ocva2, Pitpnm3, 4930563E22Rik, 4933427D14Rik, 6330403K07Rik, Aspa, Cluh, E130309D14Rik, Pimreg, Haspin, Hic1, Itgae, Nlrp1a, Nlrp1b, Ocva2, Pitpnm3, 4930563E22Rik, 4933427D14Rik, 6330403K07Rik, Aspa, Cluh, E130309D14Rik, Pimreg, Haspin, Hic1, Itgae, Nlrp1a, Nlrp1b, Ocva2, Pitpnm3, 4930563E22Rik, 4933427D14Rik, 6330403K07Rik, Aspa, Cluh, E130309D14Rik, Pimreg, Haspin, Hic1, Itgae, Nlrp1a, Nlrp1b, Ocva2, Pitpnm3.
Fig. 1  Fine mapping of Dsm5 with ISC strains. A Dsm5 ISC lines carry varying 129-derived chromosome 11 segments (colors) on a congenic B6 background (black). B6.129-Dsm5 ISC lines were crossed with 129.Scn1a<sup>+/−</sup> mice and survival of resulting Scn1a<sup>+/−</sup> offspring was monitored to 8 weeks of age. B Comparison of female and male F1 controls with heterozygous 129/B6 alleles in Dsm5 showed a sex-dependent difference in survival, with females (n=80) having worse survival than males (n=84) (p<0.01, LogRank Mantel-Cox). C–D Hazard ratios for all ISC lines relative to F1 controls are plotted against –log_{10}(p-values) as determined by LogRank Mantel–Cox test, and Kaplan–Meier survival plots are shown for lines ISC-D, ISC-E and ISC-F. Survival was significantly improved in female mice with homozygous 129/129 alleles in ISC-D (p<0.001), ISC-E (p<0.05), and ISC-F (p<0.05) compared to F1.KO heterozygous controls (C), while only males with homozygous 129/129 alleles in ISC-D (p<0.05) had improved survival compared to F1 controls (D). Shaded areas on Kaplan–Meier plots are 95% confidence intervals for F1 controls with heterozygous 129/B6 alleles.

Table 1  Interval specific mapping

| Line | Interval   | Females (F1 median survival = 24) | Males (F1 median survival = 27.5) |
|------|------------|----------------------------------|----------------------------------|
|      |            | Median Survival (days) | LogRank p-value | Hazard Ratio (95% CI) | Median Survival (days) | LogRank p-value | Hazard Ratio (95% CI) |
| A    | 1 – 88.8 Mb | 25.5                | 0.9999          | 1.000 (0.5374 to 0.861) | > 56                  | 0.5600          | 1.217 (0.6454 to 2.295) |
| B    | 1– 59.3 Mb  | 25.0                | 0.4965          | 1.282 (0.6445 to 2.550) | 24                    | 0.0757          | 0.5840 (0.2671 to 1.277) |
| C    | 45.4 – 59.3 Mb | 25                | 0.8791          | 1.039 (0.6183 to 1.745) | 24                    | 0.6925          | 0.8693 (0.4034 to 1.873) |
| D    | 65.4 – 88.4 Mb | > 56               | 0.0003          | 3.287 (2.073 to 5.210) | > 56                  | 0.0428          | 2.615 (1.347 to 5.075)  |
| E    | 65.4 – 76.1 Mb | 55                 | 0.0048          | 2.444 (1.505 to 3.970) | 26.5                  | 0.7614          | 0.9110 (0.4755 to 1.745) |
| F    | 71.8 – 88.4 Mb | 54.5               | 0.0050          | 2.259 (1.422 to 3.588) | 48                    | 0.3604          | 1.360 (0.7402 to 2.497) |
Rpa1, Rtn4rl1, Smg6, Spns3, Tax1bp3, Tekt1, Trpv1, Trpv3, Xaf1, Zzef1) (Table 2). Of the 30 protein coding genes with identified coding sequence or expression differences, ten had a prior association with seizure or epilepsy based on literature and database searches, including Aspa, Hic1, Nlrp1a, Nlrp1b, Pafah1b1, Smg6, Trpv1, Trpv3, Ywhae and 6330403K07Rik (Table 2). Pathway analysis of these Dsm5 candidate genes showed that Trpv1 is the only first neighbor to Scn1a, while Trpv3 and Aspa are included in this network through their interactions with Trpv1 (Fig. 3).

Three of the brain-expressed miRNAs (miRs-22, -132, -212) had prior association as seizure-responsive genes according to EpimiRBase, a database cataloging published reports of miRNA up- and downregulation following seizures (Jimenez-Mateos et al. 2011; Mooney et al. 2016). It is notable that two of these miRNAs (miRs-132, and -212) are shown to be upregulated following seizures and are predicted to target voltage-gated sodium channel genes, including Scn1a. Furthermore, a recent study demonstrated that miR-132 regulates Nav1.1 expression in a chronic cerebral hypoperfusion model (Hu et al. 2019).

To assess whether seizures affected expression of miR-132-3p or miR-212-3p, we performed RT-ddPCR and compared expression between F1.Scnn1a+/− mice with and without recent seizures. Consistent with prior reports, we found that F1.Scnn1a+/− mice with recent seizures had elevated levels of miR-132-3p and a trend toward elevated levels of miR-212-3p relative to those that were seizure-free in the antecedent 24 h (Fig. 4). This suggests the possibility that seizure-mediated elevation of miR-132-3p and/or miR-212-3p could downregulate expression of...
Na\textsubscript{\textit{v}}1.1 and exacerbate the effect of \textit{Scn1a} heterozygous deletion.

**Discussion**

In the present study, we constructed a set of ISCs to fine map the \textit{Dsm5} locus on mouse chromosome 11. Under a single gene modifier model, our results narrow the modifier interval to a 5.7 Mb region of overlap between ISC-E and ISC-F. Within this interval, we identified a number of potential candidate modifier genes with coding sequence variation and/or expression differences between the strains. However, future studies will be needed to validate candidate modifier genes.

Among the \textit{Dsm5} potential modifier genes, \textit{Trpv1} is an intriguing candidate as a proposed target of cannabidiol, an FDA/EMA-approved Dravet syndrome therapeutic (Gray and Whalley 2020). Recently, we reported differential expression of \textit{Trpv1} transcript in cortex, with higher expression in F1.\textit{Scn1a}\textsuperscript{+/−} mice relative to seizure resistant 129.\textit{Scn1a}\textsuperscript{+/−} mice (Satpute Janve et al. 2021). However, deletion of \textit{Trpv1} had both pro- and anti-convulsant effects when combined with the \textit{Scn1a}\textsuperscript{+/−} allele. Double mutant F1.\textit{Scn1a}\textsuperscript{+/−};\textit{Trpv1}\textsuperscript{+/−} mice had lower temperature thresholds for hyperthermia-induced seizure compared to F1.\textit{Scn1a}\textsuperscript{+/−} mice, suggesting a pro-convulsant effect. Conversely, \textit{Trpv1} deletion resulted in reduced severity of spontaneous GTCS in double mutant F1.\textit{Scn1a}\textsuperscript{+/−};\textit{Trpv1}\textsuperscript{+/−} mice, although it did not affect seizure frequency or survival compared to F1.\textit{Scn1a}\textsuperscript{+/−} mice (Satpute Janve et al. 2021). Although \textit{Trpv1} deletion alters seizure phenotypes in \textit{Scn1a}\textsuperscript{+/−} mice, it is not possible to separate an effect of \textit{Trpv1} deletion from residual 129 alleles in the chromosome 11 region remaining from the homologous

| Table 2 | Single-gene model positional candidate protein coding genes with expression and/or coding differences between B6 and 129 |
|---------|---------------------------------------------------------------------------------------------------------------|
| Gene    | NCBI Gene ID | Chromosome 11 Location (GRCh38.p6) | Expression difference | Coding difference | Prior association with epilepsy and/or Seizures |
| 6330403K07Rik | 103,712 | 71,031,941–71,033,513 | WT 129 v WT F1 | Yes | Yes |
| \textit{Nlrp1a} | 195,046 | 71,092,236–71,144,704 | Yes | Yes |
| \textit{Nlrp1b} | 637,515 | 71,153,102–71,230,733 | Yes | Yes |
| \textit{Pimreg} | 109,212 | 72,042,032–72,047,378 | Yes | Yes |
| \textit{Pitpnm3} | 327,958 | 72,047,528–72,135,778 | Yes | Yes |
| 4933427D14Rik | 74,477 | 72,153,929–72,207,459 | Yes | |
| 4930563E22Rik | 75,304 | 72,215,138–72,218,444 | Yes | |
| \textit{Xaf1} | 327,959 | 72,301,629–72,313,733 | Yes | |
| \textit{Tek1} | 21,689 | 72,344,722–72,362,442 | Yes | |
| \textit{Snp3} | 77,577 | 72,494,919–72,550,506 | Yes | |
| \textit{Zef1} | 195,018 | 72,796,226–72,927,120 | Yes | |
| \textit{Itgae} | 16,407 | 73,090,583–73,147,446 | Yes | |
| \textit{Haspin} | 14,841 | 73,135,485–73,138,294 | Yes | |
| \textit{Taxibp3} | 76,281 | 73,177,083–73,183,162 | Yes | |
| \textit{Trpv1} | 193,034 | 73,234,292–73,261,242 | Yes | Yes |
| \textit{Trpv3} | 246,788 | 73,267,388–73,300,363 | Yes | Yes |
| \textit{Aspa} | 11,484 | 73,304,992–73,329,596 | Yes | Yes |
| \textit{E130309D14Rik} | 432,582 | 74,619,605–74,641,516 | Yes | |
| \textit{Clu} | 74,148 | 74,649,495–74,670,847 | Yes | |
| \textit{Pafah1b1} | 18,472 | 74,673,949–74,724,670 | WT 129 vs WT F1 | Yes |
| \textit{Sgs2} | 97,761 | 74,849,261–74,897,060 | WT 129 vs WT B6 (Females) | |
| \textit{Smg6} | 103,677 | 74,925,823–75,164,448 | WT 129 vs WT B6 (Females) | Yes | Yes |
| \textit{Hic1} | 15,248 | 75,164,565–75,169,519 | Yes | Yes |
| \textit{Ovet2} | 246,257 | 75,175,942–75,178,835 | Yes | |
| \textit{Rtn4r1l} | 237,847 | 75,193,783–75,267,769 | F1 KO v F1 KO with recent seizures | Yes |
| \textit{Rpa1} | 68,275 | 75,298,166–75,348,324 | F1 KO v F1 KO with recent seizures | Yes |
| \textit{Smyd4} | 319,822 | 75,348,433–75,405,705 | F1 KO v F1 KO with recent seizures | Yes |
| \textit{Serpinf1} | 20,317 | 75,409,769–75,422,701 | F1 KO v F1 KO with recent seizures | Yes |
| \textit{Ywhae} | 22,627 | 75,732,869–75,765,845 | WT 129 vs WT F1 | Yes |
recombination in JM-1 ES cells (129X1/SvJ) (Caterina et al. 2000). Lack of any effect of a Trpv1-selective antagonist on F1.Scn1a<sup>+/−</sup> phenotypes lends support for an effect of residual 129 alleles rather than Trpv1 deletion itself on Scn1a<sup>+/−</sup> seizure phenotypes (Satpute Janve et al. 2021). Additional intriguing candidates include the miRNA genes for miR-132-3p and miR-212-3p that are predicted to target Scn1a and are seizure-responsive genes. However, to date, we have no evidence for altered expression of Scn1a transcript between strains or in response to seizures. It is possible that miRNA regulation could be post-transcriptional and alter protein levels.

The Dsm5 region overlaps previously identified seizure susceptibility QTL, including Szs10 and Gss4 (Ferraro et al. 2001; Gu et al. 2020). In addition, this region of mouse chromosome 11 is syntenic with recurrent CNVs implicated in epilepsy and neurodevelopmental disorders (Coppola et al. 2019; Kolishovski et al. 2019). This includes synteny with Miller-Dieker lissencephaly 17p13.3 microdeletion syndrome, which includes epilepsy as part of the neurological features. The 17p13.3 microdeletion includes deletion of PAFAH1B1 and YWHAE, as well as deletion of the intervening genes. The lissencephaly phenotype is attributed to deletion of PAFAH1B1, formerly known as LIS1, while disruption of YWHAE, encoding 14–3-3ε, is associated with variable structure brain abnormalities, cognitive impairment and seizures (Cardoso et al. 2000; Noor et al. 2018; Romano et al. 2020). Rare microduplications at 17q12 are associated with epilepsy, including familial FS/GEFS + that is also often caused by SCN1A variants (Hardies et al. 2013;
Mefford et al. 2016, 2007). Genes associated with the 17q12 3'UTR. F1. Scn1a seed match sites in the +/- mice with recent epilepsy effects. Additional support for this hypothesis comes from males that showed robust improvement in survival with ISC-D, whereas ISC-E or ISC-F alone had no protective effect. Ideally, these two possibilities could be tested empirically by generation of ISC lines separating the E–F overlapping and non-overlapping regions; however, such recombination events have been elusive in our ISC colony. It may be possible in the future to use genome engineering to further dissect the interval.

Haploinsufficiency for SCN1A is a major cause of Dravet syndrome, and yet there is variable expressivity among patients with this shared genetic basis. This suggests that clinical presentation is influenced by other factors, which may include genetic modifiers. Identification of modifier genes that influence disease course will provide insights into understanding the molecular basis of Dravet syndrome and identify potential pathways for intervention.

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Author Contributions Conceptualization: JAK; Formal analysis: JAK; Investigation: JAK, LDCH, NAH, SD, NAZ, IKE; Methodology: NAH, LDCH, JAK; Project administration: JAK; Supervision: JAK; Visualization: JAK; Writing – original draft: JAK; Writing – review & editing: LDCH, SD, NAZ, IKEF, NAH, JAK; Funding acquisition: JAK.

Data availability Genomic and transcriptomic datasets generated during and/or analyzed during the current study are available in the NCBI GEO repository [GSE112627] and SRA repository [PRJNA817075]. Other datasets are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest JAK serves as a consultant to Encoded Genomics, Praxis Precision Medicines and NeuroCycle Therapeutics, and serves on the Scientific Advisory Boards of the Dravet Syndrome Foundation and FamilieSCN2A Foundation. JAK receives research funding from Praxis Precision Medicines and Pfizer. NAH serves as a consultant to NeuroCycle Therapeutics. All other authors have declared that no competing interests exist.

Ethical approval 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.

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