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# GABRA1 and STXBP1: Novel genetic causes of Dravet syndrome

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**Supplemental data  
at Neurology.org**

## ABSTRACT

**Objective:** To determine the genes underlying Dravet syndrome in patients who do not have an *SCN1A* mutation on routine testing.

**Methods:** We performed whole-exome sequencing in 13 *SCN1A*-negative patients with Dravet syndrome and targeted resequencing in 67 additional patients to identify new genes for this disorder.

**Results:** We detected disease-causing mutations in 2 novel genes for Dravet syndrome, with mutations in *GABRA1* in 4 cases and *STXBP1* in 3. Furthermore, we identified 3 patients with previously undetected *SCN1A* mutations, suggesting that *SCN1A* mutations occur in even more than the currently accepted ~75% of cases.

**Conclusions:** We show that *GABRA1* and *STXBP1* make a significant contribution to Dravet syndrome after *SCN1A* abnormalities have been excluded. Our results have important implications for diagnostic testing, clinical management, and genetic counseling of patients with this devastating disorder and their families. *Neurology*® 2014;82:1245-1253

## GLOSSARY

**cdNA** = complementary DNA; **dHPLC** = denaturing high-performance liquid chromatography; **FS** = febrile seizures; **GABA** =  $\gamma$ -aminobutyric acid; **GEFS+** = genetic epilepsy with febrile seizures plus; **WES** = whole-exome sequencing; **WT** = wild-type.

Dravet syndrome (Online Mendelian Inheritance in Man #607208), previously known as severe myoclonic epilepsy of infancy, is an infantile-onset epileptic encephalopathy characterized by a distinctive electroclinical and developmental course culminating in intellectual disability and refractory seizures. The genetic basis of this disorder is attributed to heterozygous disease-causing mutations in the sodium channel  $\alpha 1$  subunit gene, *SCN1A*, in 75% of patients; 90% of mutations arise de novo.<sup>1,2</sup> A small proportion of girls and one mosaic male, with a phenotype resembling Dravet syndrome, have mutations of protocadherin 19, *PCDH19*.<sup>3,4</sup> Two patients with heterozygous truncating *GABRG2* mutations and 2 case reports with homozygous *SCN1B* mutations have also been described.<sup>5-8</sup> Finally, recently, 3 patients with de novo *CHD2* mutations and several overlapping features of Dravet syndrome were reported.<sup>9</sup> These mutations, however, are rare, and the genetic etiology of most patients with Dravet syndrome without mutations in *SCN1A* remains to be solved. Here we employ a whole-exome sequencing (WES) and targeted resequencing approach for gene discovery in *SCN1A*-negative patients with Dravet syndrome.

**METHODS Standard protocol approvals, registrations, and patient consents.** Informed consent was obtained from all patients and in the case of minors, their parents or legal guardians. This study was approved by the human research ethics committees at Austin Health, the University of Washington, and the Christian-Albrechts University, as well as the Commission for Medical Ethics at the University of Antwerp.

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Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

**Patients: WES cohort.** Proband with Dravet syndrome were recruited from the epilepsy clinic at Austin Health, from the practices of the investigators, and by referral for epilepsy genetics research from Australia and New Zealand. A diagnosis of Dravet syndrome was based on the following criteria: onset less than 15 months of age with convulsive seizures (hemiclonic or generalized) that were often prolonged and triggered by fever. Other seizure types emerged over time, including focal, myoclonic, absence seizures, and drop attacks. Development was normal in the first year of life with later slowing and intellectual disability.

The 13 patients subject to WES had been previously screened for *SCN1A* point mutations using denaturing high-performance liquid chromatography (dHPLC) (n = 4) or bidirectional sequencing (n = 9). Small exonic deletion/duplications had also been excluded using *SCN1A* multiplex ligation-dependent probe amplification and all patients were negative for large copy number variants (reference<sup>10</sup> and unpublished data).

**WES and analysis.** The exome sequencing libraries of 34 individuals, including 10 parent–proband trios, 1 mother–proband pair, and 2 unrelated probands were prepared using the SeqCap EZ Human Exome Library v2.0 (Roche, Nimblegen). Libraries were sequenced on an Illumina HiSeq, using a 50 bp paired-end read protocol as per the manufacturer's recommendations. Reads were aligned to the human genome (hg19) using the Burrows-Wheeler Aligner,<sup>11</sup> removing all potential PCR duplicates. The Genome Analysis Toolkit<sup>12</sup> was used for base quality recalibrations, realignment around known indels, variant calling, and filtering to retrieve only high-quality variants. We considered only rare, disruptive (missense, nonsense, splice, frameshift) variants that were not present in the ESP6500 control dataset (see URLs in the appendix) for further analysis.

**Patients: Targeted resequencing (WES) cohort.** We performed targeted resequencing of candidate genes in a cohort of 67 Dravet and Dravet-like patients. All 67 of these patients had been screened for *SCN1A* mutations previously by the various collaborating institutions. In addition, we performed *SCN1A* mutation screening using molecular inversion probes and high-throughput sequencing. Only *SCN1A*-negative patients were included in the validation cohort (n = 67).

**Targeted resequencing of candidate genes.** We selected 15 candidate genes (*STXBPI*, *GABRA1*, *SCN1B*, *ATP6VOC*, *SLC8A1*, *CLSNT1*, *NKAIN3*, *NOL11*, *RIMS2*, *KIF1B*, *CDK5RAP3*, *ABTB2*, *STK31*, *KDM2B*, *SPATA13*) from the WES analysis for mutation screening in a validation cohort of 67 *SCN1A*-negative Dravet probands. From the 13 cases in whom WES was performed, we identified candidate genes belonging to one of 3 categories, based on the presence of a rare variant in that gene. Three candidate genes (*STXBPI*, *GABRA1*, *SCN1B*) were previously implicated in epileptic encephalopathies or other epilepsies. An additional 5 genes (*ATP6VOC*, *SLC8A1*, *CLSNT1*, *NKAIN3*, *NOL11*) were selected as candidates as we identified a rare, de novo variant in a single proband. Finally, we selected 7 genes with variants that segregated in a recessive manner in a single proband (*RIMS2*, *KIF1B*, *CDK5RAP3*, *ABTB2*, *STK31*, *KDM2B*, *SPATA13*). We used molecular inversion probes to “capture” exonic regions and 5 flanking intronic base pairs of target genes, and performed massively parallel sequencing and variant detection as described previously.<sup>13,14</sup>

**GABA<sub>A</sub> mutagenesis and in vitro transcription.** Human GABA<sub>A</sub> complementary DNA (cDNA) was cloned into the pGEMHE vector containing a T7 promoter for in vitro transcription. The Gly251Ser mutation was generated using QuikChange Site-

Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA) with primers forward 5'-GAAGAGAAAGATTAGCTACTTTGTTATTC AACATACCTGCGC and reverse 5'-GGCAGGTATGTTTGAATAACAAAGTAGCTAATCTTTCTCTTC. Gly251Ser mutation is underlined. The GABRA1 (Gly251Ser) pGEMHE plasmid was verified by DNA sequencing. cRNA was made using linearized cDNA template and in vitro transcription performed using the mMessage mMachine kit (Applied Biosystems/Ambion, Austin, TX).

#### **GABA modulation of wild-type and mutant receptors.**

Oocytes from adult female *Xenopus laevis* were prepared as previously described.<sup>6</sup> Fifty nanoliters of cRNA encoding the wild-type (WT) human A1, B2, and G2L and mutant A1 (Gly251Ser) GABA receptor subunits (12 ng/μL; stocks confirmed spectrophotometrically and by gel analysis) were injected into the cytoplasm of stage 5 or 6 oocytes using the Roboocyte Robot (Multi Channel Systems, Reutlingen, Germany) and stored for 2 days prior to experimentation. Two-electrode voltage clamp recordings were made in 96-well plates using the Roboocyte automated platform. Oocytes were impaled using recording heads with 2 glass electrodes containing 1.5 M potassium acetate and 0.5 M KCl and held at a membrane potential of -80 mV. Oocytes were continually perfused with a ND96 solution (96 mM NaCl, 2 mM KCl, 0.1 mM CaCl<sub>2</sub>, and 5 mM HEPES, pH 7.5) using a Gilson 222 XL Liquid Handler and Gilson Minipuls 3 Peristaltic Pump (Gilson Medical Electronics, Middleton, WI). To construct a dose-response curve, oocytes were exposed to a 30-second application of test γ-aminobutyric acid (GABA) (Sigma Aldrich, Sydney, Australia) (range 1 μM–1 mM) followed by a 60-second wash in ND96 and then a 15-second application of a maximum dose of GABA (1 mM). Only 1 test concentration and 1 maximum concentration of GABA was applied per oocyte. The effect of the test GABA concentration on an individual oocyte was expressed as a percentage of the maximal GABA response in the same oocyte. These percentages were then averaged from many oocytes (range 8–20 oocytes per test dose). Maximum current at 1 mM GABA was also averaged over many oocytes (100 for WT, 97 for Gly251Ser mutation).

#### **RESULTS**

We performed WES in 13 *SCN1A*-negative Dravet syndrome probands (clinical features in table 1), including 10 parent–proband trios, 1 mother–proband pair, and 2 unrelated probands, to identify novel genetic causes for this devastating disorder. On average, we generated 3.8 Gb of mapped sequence data per individual and 92% of bases had >8× coverage across all samples. On average, ~27,000 raw variants were identified in each individual. We prioritized only disruptive (nonsynonymous, splice, frameshift) variants that were not present in the ESP6500 control dataset (see URLs in the appendix) for further analysis and initially applied a de novo model for gene discovery in these patients.

**De novo variants.** Under a de novo disease model, we identified 15 rare, disruptive variants in 9 individuals, including 2 individuals (T1895, T1911) who were originally sequenced as singletons and whose mutations were confirmed as occurring de novo using Sanger sequencing in the parents (table 2). Five of these de novo variants occurred in known epilepsy genes. Unexpectedly, 3 variants were detected in *SCN1A* that were not previously

**Table 1** Clinical features of patients with Dravet syndrome who underwent whole-exome sequencing or in whom mutations were identified by targeted resequencing

Patient	Age, y/ sex	Seizure onset age (mo), seizure type	Seizure types	Fever sensitivity	Intellect (regression)	EEG	MRI	Likely causative gene	Testing method
T20744	2/F	8, Brief H	FDS, H, SE, TCS	Present	Mild delay (no)	Normal	Normal	GABRA1	WES
T16706	7/F	11, Febrile, 20 min TCS	Ab, FDS, H, Myo, TCS	Present	Moderate ID (yes)	GSW	Normal	GABRA1	Targeted resequencing
T23532	18/M	11, Febrile, 10 min H	Ab, At, FDS, H, SE, T, TCS	Present	Moderate ID (yes)	Focal discharges	Calcified subependymal nodule in left lateral ventricle	GABRA1	Targeted resequencing
Co05	18/M	8, H SE	Ab, At, FDS, H, Myo, SE, TCS	Present	Mild ID (unknown)	GSW, MFD, PPR	Normal	GABRA1	Targeted resequencing
T1915	11 <sup>a</sup> /M	11, Afebrile cluster of TCS	At, FDS, Myo, SE, T, TCS	Present	Severe ID, deceased aged 11 y (yes)	MFD	Normal	STXBP1	WES
EP1807	21/M	6, Febrile FDS	Ab, FDS, Myo, TCS	Present	Severe ID (yes)	MFD	Atrophy	STXBP1	Targeted resequencing
T21717	6/F	12, Brief febrile TCS	Ab, At, H, Myo, T, TCS	Present	Learning difficulties (no)	Normal	ND	STXBP1	Targeted resequencing
T888	23/F	6, Afebrile H SE	Ab, FDS, H, Myo, SE, TCS	Present	Moderate ID (no)	GSW, PPR	Normal	SCN1A	WES
T1895	17 <sup>a</sup> /M	11, Febrile SE	aAb, At, FDS, Myo, NCS, SE, TCS	Present	Severe ID, deceased aged 20 (yes)	GSW, PPR, MFD	Normal	SCN1A	WES
T17775	7/F	3, 10 min afebrile TCS	Ab, At, FDS, H, Myo, NCS, SE, TCS	Present	Severe ID (yes)	GSW, PSW, MFD	Normal	SCN1A	WES
T22809	3/M	6, Febrile SE	Ab, Myo, SE, TCS	Present	Mild ID (yes)	Normal	Normal	SCN1B	WES
T20038	10/F	6, Brief febrile TCS	TCS	Present	Mild ID (no)	Normal	Normal	None	WES
T16860	26/M	2, Brief TCS	Ab, At, FDS, Myo, SE, TCS	Present	Mild ID (unknown)	Focal discharges	Cerebellar atrophy	None	WES
T1911	8/M	7, TCS	At, FDS, Myo, T, TCS	Present	Severe ID (yes)	GSW, MFD	Normal	None	WES
T3892	9/M	4, Febrile SE	Myo, FDS, H, SE, TCS	Present	Moderate ID (no)	GSW, PSW, MFD	Normal	None	WES
T863	11/F	6, Ab	Ab, At, H, Myo, NCS, SE, T, TCS	Present	Mild ID (yes)	Normal	Delayed myelination	None	WES
T19264	9/F	14, Febrile TCS	Ab, FDS, H, Myo, SE, TCS	Present	Severe ID (no)	GSW, PPR	Normal	None	WES
T2985	39/M	6, Febrile, 15 min H	At, FDS, H, Myo, SE, TCS	Present	Moderate ID (no)	GSW, MFD	Normal	None	WES

Abbreviations: aAb = atypical absence; Ab = absence; At = atonic; FDS = focal dyscognitive seizures; GSW = generalized spike-wave; H = hemiclonic; ID = intellectual disability; MFD = multifocal discharges; Myo = myoclonic; NCS = nonconvulsive status; ND = not done; PPR = photo-paroxysmal response; PSW = polyspike wave; SE = status epilepticus; T = tonic; TCS = tonic-clonic seizure; WES = whole-exome sequencing.

<sup>a</sup>Deceased.

**Table 2** Rare, disruptive variants of interest in 13 patients with Dravet syndrome detected by WES and 67 patients by candidate gene targeted resequencing

Proband	Gene	Inheritance	cDNA change	GERP	Amino acid change	Polyphen score	ESP control allele frequency (%)
<b>Probands with mutations in known epilepsy genes</b>							
T888	<i>GAS2L2</i>	De novo	c.2347G>A	2.93	Arg783Trp	0.99	Not present
	<i>SCN1A</i>	De novo	c.2044-1G>A	5.47	Unk	NA	Not present
	<i>IGSF8</i>	De novo	c.82G>A	4.9	Arg27Trp	1	Not present
T17775	<i>SCN1A</i>	De novo	c.383C>A	4.72	Ser128 <sup>a</sup>	NA	Not present
T1895 <sup>a</sup>	<i>SCN1A</i>	De novo	c.1738G>A	4.19	Arg580 <sup>a</sup>	NA	Not present
T1915 <sup>a</sup>	<i>STXBP1</i>	De novo	c.847G>A	5.32	Glu283Lys	0.97	Not present
T20744	<i>GABRA1</i>	De novo	c.751G>A	5.05	Gly251Ser	1	Not present
T23532 <sup>c</sup>	<i>GABRA1</i>	De novo	c.335G>A	5.85	Arg112Gln	0.83	Not present
T16706 <sup>c</sup>	<i>GABRA1</i>	Unk <sup>d</sup>	c.335G>A	5.85	Arg112Gln	0.83	Not present
Co05 <sup>c</sup>	<i>GABRA1</i>	De novo	c.917A>C	5.47	Lys306Thr	0.99	Not present
T21717 <sup>c</sup>	<i>STXBP1</i>	De novo	c.853G>T	5.95	Asp285Tyr	1	Not present
EP1807 <sup>c</sup>	<i>STXBP1</i>	De novo	c.1334A>C	5.2	His445Pro	0.03	Not present
T22809	<i>SCN1B</i>	Maternal	c.363C>G	2.49	Cys121Trp	1	0.02
<b>Probands with mutations in candidate genes</b>							
T1911	<i>NKAIN3</i>	De novo	c.216G>T	5.53	Trp72Cys	1	Not present
	<i>ATP6VOC</i>	De novo	c.133_134delCT	4.85	Ser45Cysfs*37	NA	Not present
	<i>SLC8A1</i>	De novo	c.2888G>C	5.48	Cys963Ser	1	Not present
T16860	<i>NOL11</i>	De novo	c.144+1G>A	4.86	Unk	NA	Not present
T19264	<i>FARP2</i>	De novo	c.964C>G	4.32	Leu322Val	0.99	Not present
	<i>COL6A3</i>	De novo	c.8763_8764insT	-	Pro2922Thrfs*10		
T20038	<i>CLSTN1</i>	De novo	c.2607C>A	4.71	Ser869Arg	0.98	Not present
	<i>CYP26C1</i>	De novo	c.731A>G	5.16	His244Arg	0.42	Not present
T2985 <sup>b</sup>	WES sample with no de novo mutations in known or candidate genes						
T3892	WES sample with no de novo mutations in known or candidate genes						
T863	WES sample with no de novo mutations in known or candidate genes						

Abbreviations: cDNA = complementary DNA; GERP = genomic evolutionary rate profiling; NA = not available; Unk = unknown; WES = whole-exome sequencing. All amino acid changes resulting from frameshift mutations are predicted using Mutalyzer (see URLs in the appendix).

<sup>a</sup>These probands were not sequenced as part of a trio but rather as singletons; the de novo nature of the variant was confirmed by Sanger DNA sequencing.

<sup>b</sup>Proband was sequenced as mother-child pair; remaining probands were all sequenced as part of a proband-parent trio.

<sup>c</sup>All variants were detected by targeted resequencing.

<sup>d</sup>Parents unavailable to determine variant segregation.

identified by Sanger DNA sequencing in 2 and dHPLC in the third. Furthermore, we detected a single mutation in 2 genes previously implicated in other epilepsy syndromes, *GABRA1* and *STXBP1* (table 2). We identified 3 additional probands with *GABRA1* mutations (figure 1A) and 2 patients with de novo *STXBP1* mutations by targeted resequencing in 67 patients with a clinical diagnosis of Dravet syndrome (table 2).

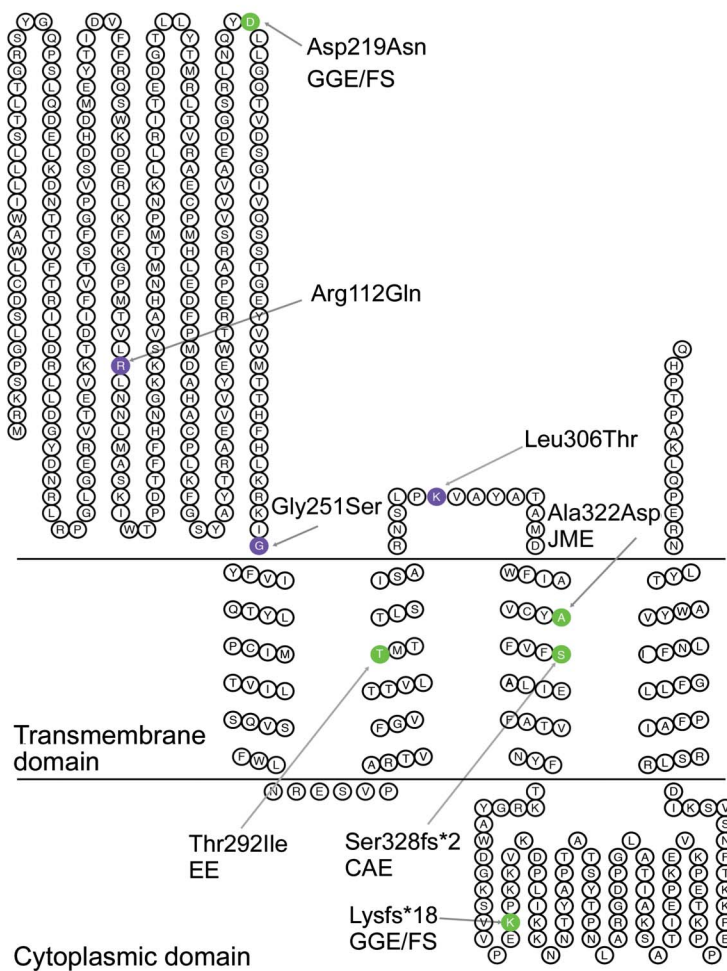
Of the 10 trios who underwent WES, 3 probands had no candidate de novo mutations that passed our filtering criteria, whereas 4 subjects had de novo mutations in one or more genes that are not known epilepsy genes (table 2). Each gene was only implicated

in 1 patient, with unique de novo events in 8 genes. In order to validate these genes in Dravet syndrome, we prioritized 5 candidate genes (*ATP6VOC*, *SLC8A1*, *CLSTN1*, *NKAIN3*, *NOL11*) for further study. We excluded *FARP2*, *COL6A3*, and *CYP26C1* as candidate genes given that they encode proteins with no obvious neuronal function or had multiple putatively truncating mutations in the ESP control dataset. Targeted resequencing in these 5 candidate genes in the validation cohort (n = 67) revealed no additional rare, de novo pathogenic variants.

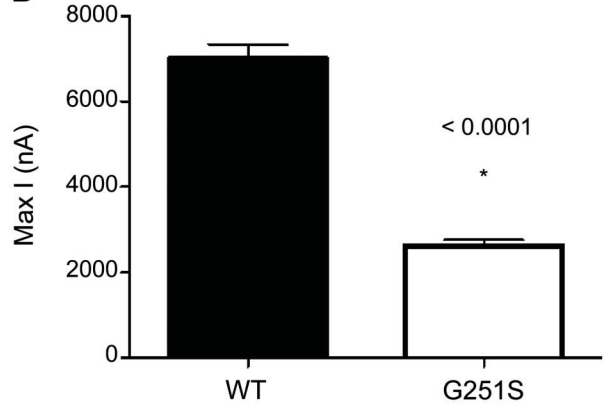
**Inherited mutations in known epilepsy genes.** We identified all disruptive variants in known epilepsy genes

**Figure 1** GABRA1 mutations in epilepsy and effects on  $\gamma$ -aminobutyric acid (GABA) response

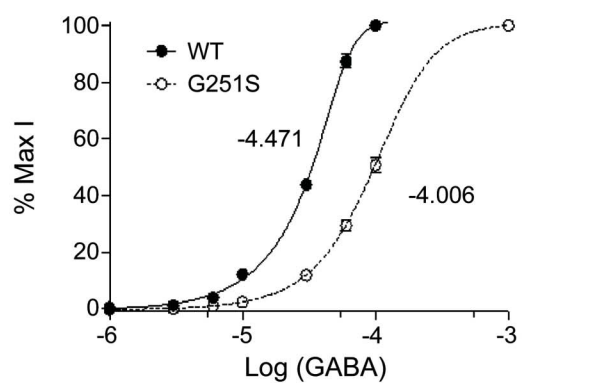
**A Extracellular domain**



**B**



**C**



(A) The amino acid locations of the 8 mutations identified in patients with Dravet syndrome (purple) and other epilepsy syndromes (green). There is no clear genotype-phenotype correlation with respect to either nature or localization of the mutation and severity of phenotype. (B) Maximal current response (1 mM GABA) of the wild-type (WT) and p.Gly251Ser mutant. (C) GABA dose-response curves of the WT and p.Gly251Ser mutant in *Xenopus laevis* oocytes. CAE = childhood absence epilepsy; EE = epileptic encephalopathy; GGE/FS = genetic generalized epilepsies/febrile seizures; JME = juvenile myoclonic epilepsy.

with an allele frequency in the ESP dataset of <math>< 1\%</math> in the 8 patients with Dravet syndrome who underwent WES, but did not carry de novo mutations in known epilepsy genes (table e-1 on the *Neurology*<sup>®</sup> Web site at Neurology.org). Of interest, we detected a maternally inherited c.363C>T (p.Cys121Trp) in *SCN1B* in T22809; this individual had no candidate de novo mutations (figure e-1). This mutation has been described in families with other types of epilepsy.<sup>15,16</sup>

**Autosomal recessive model for Dravet syndrome.** Given that 2 recessive cases of Dravet syndrome have been reported,<sup>7,8</sup> we applied this disease inheritance model in the 7 probands without mutations in known epilepsy genes and identified 15 genes with variants that followed an autosomal recessive pattern (variant allele frequency <math>< 1\%</math>) (table e-2). Targeted resequencing was performed in 7 candidate genes (*RIMS2*, *KIF1B*, *CDK5RAP3*, *ABTB2*, *STK31*, *KDM2B*, and

*SPATA13*). We excluded the remaining genes as they have been implicated in unrelated disorders (*MLL2*, *PDE6B*, *PCNT*) or have no known or obvious neuronal function (*VWA5B2*, *OAS3*, *DCHS2*, *DNAH3*, *DNAH11*). We found no instances of autosomal recessive inheritance in our validation cohort ( $n = 67$ ).<sup>7,8</sup>

**Dose response of the GABRA1 mutant p.Gly251Ser to GABA.** To assess the effect of the p.Gly251Ser GABA<sub>A</sub> mutation on neuronal function, we measured GABA-mediated currents in *X laevis* oocytes expressing mutant (p.Gly251Ser) GABA<sub>A</sub> (e-Methods). Maximum current values recorded at -1 mM GABA dosage showed a 2.6-fold reduction in the amplitude of GABA-induced currents in vitro for the p.Gly251Ser mutant (max I  $\pm$  SEM: 2,621  $\pm$  142,  $n = 97$ ) compared to WT (max I  $\pm$  SEM: 7,010  $\pm$  325,  $n = 100$ ) (figure 1B). Furthermore, the GABA dose-response curves showed a 5-fold decrease in

sensitivity to GABA of the p.Gly251Ser mutant compared to WT (figure 1C).

**DISCUSSION** We applied massively parallel sequencing approaches in 67 *SCN1A*-negative patients with Dravet syndrome to identify novel de novo genetic causes for this devastating disorder. Overall, we show that *GABRA1* and *STXBPI* are new causes for Dravet syndrome. Furthermore, we identified 3 patients with undetected *SCN1A* mutations, despite previous mutation screening. This finding verifies the efficacy of our WES approach in gene discovery and the accuracy of clinical diagnosis. Furthermore, we propose that Dravet syndrome is due to mutations in *SCN1A* more often than the generally reported estimate of 75%,<sup>1</sup> as some mutations pass undetected using conventional mutation detection techniques.

Overall, we identified 4 novel *GABRA1* mutations in patients with Dravet syndrome. The clinical presentation was typical for Dravet syndrome, with the only uncommon feature being atonic drop attacks in 2 of the 4 cases. Three of the 4 variants arose de novo, while the inheritance of the fourth variant, a c.335 G>A, p.Arg112Gln in proband T16706, could not be determined as parents were unavailable. However, this same mutation (p.Arg112Gln) arose de novo in another proband (T23532), suggesting this is a recurrent pathogenic mutation resulting in Dravet syndrome. This finding redefines *GABRA1* from a gene associated with mild genetic generalized epilepsies and febrile seizures<sup>17–19</sup> to a gene also implicated in severe epilepsies such as Dravet

syndrome (figure 1A). Our results are supported by the recent identification of a de novo mutation in *GABRA1* in a patient with epileptic encephalopathy.<sup>20</sup>

We propose that the p.Gly251Ser *GABRA1* mutation reduces the ability of mutant receptors to contribute to phasic inhibition as demonstrated by the decreased sensitivity to GABA and a significantly reduced amplitude of GABA-induced currents. It is likely that the additional 2 missense *GABRA1* mutations (p.Arg112Gln, Lys306Thr) act in a similar fashion. Overall, these studies suggest that seizures in these patients are the result of impaired functioning of GABA inhibition in the brain.

We also describe 3 Dravet syndrome patients with de novo missense mutations in *STXBPI*. Our 3 patients had a Dravet phenotype with onset in the first year of life; however, 2 had both tonic and atonic seizures. Both seizure types are rare early in Dravet syndrome, although tonic seizures are reported in older patients.<sup>21</sup> Status epilepticus was only seen in 1 case. Heterozygous de novo *STXBPI* mutations cause early-onset epileptic encephalopathies and neurodevelopmental disorders. Of the >50 patients with *STXBPI* encephalopathy described, the majority present by 3 months of age, with Ohtahara syndrome or other early-onset epileptic encephalopathies.<sup>22–32</sup> Our patients had onset from 6 to 12 months, which is later than usually seen in *STXBPI* encephalopathy. It is typically associated with epileptic spasms, and notably these were not observed in our patients with Dravet syndrome. The wide range of *STXBPI* mutations show no genotype–phenotype correlation with respect to mutations and clinical presentation (figure e-2).

*STXBPI* plays a role in the release of neurotransmitters into the synapse, via regulation of syntaxin. The 3 de novo missense mutations described here all lead to the replacement of a charged residue with a neutral amino acid. These alterations are likely to destabilize the *STXBPI* protein or affect binding to syntaxin, as has been shown previously for the Cys180Tyr missense mutation,<sup>22</sup> though experimental validation needs to be performed.

We identified no de novo mutations in proband T22809; however, this individual was shown to carry a maternally inherited c.363C>T (p.Cys121Trp) in *SCN1B*. This mutation has been described in genetic epilepsy with febrile seizures plus (GEFS+) in 41 affected individuals from 4 families. The affected individuals displayed heterogeneous epilepsy phenotypes ranging from mild (febrile seizures [FS]) to moderate (temporal lobe epilepsy) and severe (epilepsy with myoclonic-atonic seizures).<sup>15,16</sup> GEFS+ families may include individuals with Dravet syndrome who also inherit a dominant familial mutation.<sup>33</sup> The p.Cys121Trp mutation affects a highly conserved residue and putatively disrupts a disulfide bridge in the

### Comment: Dravet syndrome—“Old gene,” novel mechanism

Dravet syndrome (DS, Online Mendelian Inheritance in Man#607208), or severe myoclonic epilepsy in infancy, is one of the most severe types of genetic epilepsy. Individuals with DS face a high risk of sudden unexpected death in epilepsy. In ≥75% of cases, DS is associated with mutations of the gene encoding the  $\alpha 1$  subunit of the sodium channel, *SCN1A*. However, the genetic causes of DS without mutations in *SCN1A* remain largely unknown.

Carvill et al.<sup>1</sup> performed whole-exome sequencing in 13 *SCN1A*-negative DS candidates and targeted resequencing in 67 additional patient candidates to discover novel genes underlying DS other than *SCN1A*. They identify 2 novel genes, *GABRA1* and *STXBPI*, that have an association with DS; *GABRA1* and *STXBPI* mutations have been reported in other epilepsies but not in DS. Furthermore, the authors examined and characterized one *GABRA1* mutation, p.Gly251Ser, using in vitro electrophysiology techniques. Compared to wild-type, the p.Gly251Ser mutation showed substantial reduction of sensitivity to  $\gamma$ -aminobutyric acid. These in vitro functional studies supported their genetic findings that *GABRA1* mutation causes DS. Overall, this study presents novel genetic mutations of DS, providing insights for developing new diagnostic testing and drug targets and possibly leading to individualized therapeutic strategies for DS patients with different genotypes.

1. Carvill GL, Weckhuysen S, McMahon JM, et al. *GABRA1* and *STXBPI*: novel genetic causes of Dravet syndrome. *Neurology* 2013;82:1245–1253.

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extracellular domain of the protein. p.Cys121Trp mutants induce a hyperexcitable state in vitro.<sup>34</sup> The p.Cys121Trp *SCN1B* mutation potentially contributes to the presentation of Dravet syndrome in proband T22809 and causes the FS in his mother. Interestingly, it was nonpenetrant in his maternal grandmother, which is in keeping with the low penetrance observed in GEFS+ families.<sup>35</sup> Other unaffected *SCN1B* c.363C>T (p.C121W) carriers (n = 6) have been reported,<sup>16</sup> and the variant is present in controls, suggesting other genetic or nongenetic factors modify the epilepsy phenotype. These observations recapitulate those seen in other patients with Dravet syndrome, where ~3–5% of cases have inherited a pathogenic *SCN1A* variant, typically from a more mildly affected parent with GEFS+.<sup>36</sup> Recently, 2 reports of recessive *SCN1B* mutations causing Dravet syndrome have been published, although one had an atypical phenotype,<sup>37</sup> but no heterozygous dominant mutations have been reported.<sup>7,8</sup> Our patient did not carry additional mutations in *SCN1B*, nor did we identify additional *SCN1B* mutations in our validation cohort (n = 67) by targeted resequencing of the gene. Collectively, these results suggest that *SCN1B* may play a susceptibility role in the pathogenesis of Dravet syndrome, though further investigations are required.

We show that the genetic etiology of *SCN1A*-negative Dravet syndrome can, in part, be attributed to de novo mutations in *GABRA1* and *STXBPI*. Of note, mutation screening of *GABRA1* in cohorts of patients with genetic generalized epilepsy and epileptic encephalopathies have rarely identified pathogenic mutations (data not shown and references 14, 17, 18, and 20). Our finding of 4 *GABRA1* mutations in 77 *SCN1A*-negative patients with Dravet syndrome suggests that *GABRA1* mutations may be largely limited, at least in terms of epileptic encephalopathies, to Dravet syndrome, though further studies are needed. Conversely, *STXBPI* mutations are seen in other epileptic encephalopathy phenotypes, suggesting considerable phenotypic heterogeneity compared to *GABRA1*.

*GABRA1* and *STXBPI* are significant contributors to *SCN1A*-negative Dravet syndrome that should be tested in patients with Dravet syndrome negative for *SCN1A* mutations. With identification of further cases with Dravet syndrome due to these genes, specific phenotypic patterns may emerge that distinguish these rarer causes of Dravet syndrome from those due to *SCN1A* mutations. We would argue that, in *SCN1A*-negative individuals, targeted resequencing of known epilepsy genes is a more cost-effective and high-throughput approach to diagnostic testing.

#### AUTHOR CONTRIBUTIONS

Gemma L. Carvill designed the study, performed experiments and data analysis, and wrote the manuscript. Sarah Weckhuysen performed experiments and data analysis and performed phenotypic analysis. Jacinta M. McMahon performed phenotypic analysis. Corinna Hartmann performed experiments and

data analysis. Rikke S. Møller performed phenotypic analysis. Helle Hjalgrim performed phenotypic analysis. Joseph Cook assisted with data analysis method development. Eileen Geraghty performed experiments. Brian J. O'Roak developed the MIPs methodology and analysis. Steve Petrou performed experiments and data analysis. Alison Clarke performed experiments and data analysis. Deepak Gill performed phenotypic analysis. Lynette G. Sadleir performed phenotypic analysis. Hiltrud Muhle performed phenotypic analysis. Sarah von Spiczak performed phenotypic analysis. Marina Nikanorova performed phenotypic analysis. Bree L. Hodgson performed experiments and data analysis. Elena V. Gazina performed experiments and data analysis. Arvid Suls performed experiments and data analysis. Jay Shendure developed the MIPs methodology and analysis. Leanne M. Dibbens performed experiments and data analysis. Peter De Jonghe performed phenotypic analysis. Ingo Helbig performed phenotypic analysis. Samuel F. Berkovic performed phenotypic analysis and critically reviewed the manuscript. Ingrid E. Scheffer supervised and designed the study, performed phenotypic analysis, and wrote the manuscript. Heather C. Mefford supervised and designed the study and wrote the manuscript.

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#### DISCLOSURE

G. Carvill, S. Weckhuysen, J. McMahon, C. Hartmann, R. Møller, H. Hjalgrim, J. Cook, E. Geraghty, Bree L. Hodgson, and Leanne M. Dibbens report no disclosures relevant to the manuscript. B. O'Roak is an inventor on patent PCT/US2009/30620: Mutations in contactin associated protein 2 are associated with increased risk for idiopathic autism. Steve Petrou, A. Clarke, D. Gill, L. Sadleir, H. Muhle, S. von Spiczak, M. Nikanorova, E. Gazina, and A. Suls report no disclosures relevant to the manuscript. J. Shendure is a member of the scientific advisory board or serves as a scientific consultant for Adaptive Biotechnologies, Ariosa Diagnostics, Stratos Genomics, GenePeeks, Gen9, Good Start Genetics, and Rubicon Genomics, gave expert testimony in Life Technologies v. Illumina and Johns Hopkins University v. 454 Life Sciences, and has received patent royalties from Life Technologies, Illumina, and Gen9. P. De Jonghe has research funded by the Fund for Scientific Research Flanders (FWO) and received speaker's fee from Biocodex. I. Helbig reports no disclosures relevant to the manuscript. S. Berkovic has received grant(s) from the National Health and Medical Research Council; has received honoraria from UCB; has a patent for PCDH19 testing planned; has received payment for development of educational presentations from UCB Pharma, Novartis Pharmaceuticals, Sanofi-Aventis, and Janssen Cilag; has a patent for *SCN1A* testing held by Bionomics and licensed to various diagnostic companies, with no financial return; and was a consultant to Bionomics and Athena diagnostics over 3 years ago. I. Scheffer has served on scientific advisory boards for UCB and Janssen-Cilag EMEA; serves on the editorial boards of the *Annals of Neurology* and *Epileptic Disorders*; may accrue future revenue on pending patent WO61/010176 (filed: 2008): Therapeutic Compound; has received speaker honoraria from GlaxoSmithKline, Athena Diagnostics, UCB, Biocodex, and Janssen-Cilag EMEA; has received



funding for travel from Athena Diagnostics, UCB, Biocodex, GlaxoSmithKline, and Janssen-Cilag EMEA; and receives/has received research support from the National Health and Medical Research Council of Australia, NIH, Australian Research Council, Health Research Council of New Zealand, CURE, American Epilepsy Society, US Department of Defense Autism Spectrum Disorder Research Program, the Jack Brockhoff Foundation, the Shepherd Foundation, Perpetual Charitable Trustees, and The University of Melbourne. H. Mefford has received a grant from the NIH/NINDS, Burroughs Wellcome Fund, and was a consultant for the Simons Foundation (SFARI Gene Advisory Board). Go to Neurology.org for full disclosures.

## APPENDIX

### Accession numbers

*SCN1A* (NM\_001165963.1), *STXBPI* (NM\_003165.3), *GABRA1* (NM\_000806.5), *SCN1B* (NM\_199037.3), *ATP6VOC* (NM\_001198569.1), *SLC8A1* (NM\_021097.2), *CLSTN1* (NM\_001009566.1), *NKAIN3* (NM\_173688.2), *NOL11* (NM\_015462), *RIMS2* (NM\_001100117.2), *KIF1B* (NM\_015074.3), *CDK5RAP3* (NM\_001278197.1), *ABTB2* (NM\_145804.2), *STK31* (NM\_031414.4), *KDM2B* (NM\_032590.4), *SPATA13* (NM\_001166271.1).

### Web resources

BWA-v0.5.6 (<http://bio-bwa.sourceforge.net/>)  
 GATK-v2.2-9 (<http://www.broadinstitute.org/gatk/>)  
 Seattle seq-v134 (<http://snp.gs.washington.edu/SeattleSeqAnnotation/>)  
 National Heart Lung and Blood Institute (NHLBI) Exome sequencing project (<http://evs.gs.washington.edu/EVS/>)  
 Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>)  
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 Online Mendelian Inheritance in Man (<http://www.omim.org>)

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