GABA\textsubscript{A}1 and STXBP1: Novel genetic causes of Dravet syndrome

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GABRA1 and STXBPI: Novel genetic causes of Dravet syndrome

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 STXBPI 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 STXBPI 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 = γ-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 α1 subunit gene, SCN1A, in ~75% of patients; 90% of mutations arise de novo.1,2 A small proportion of girls and one mosaic male, with a phenotype resembling Dravet syndrome, have mutations of protocadherin 19, PCDH19.3,4 Two patients with heterozygous truncating GABRG2 mutations and 2 case reports with homozygous SCN1B mutations have also been described.5–8 Finally, recently, 3 patients with de novo CHD2 mutations and several overlapping features of Dravet syndrome were reported.9 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, Seattle, Neurogenetics Group (S.W.), Department of Molecular Genetics, VIB, Antwerp, Laboratory of Neurogenetics (S.W., AS, P.D.J.), Institute Born-Bunge, University of Antwerp, Belgium; Epilepsy Centre Kempenhaeghe (S.W.), Oosterhout, the Netherlands; Epilepsy Research Centre (J.M.M., S.F.B., I.E.S.), Department of Medicine, University of Melbourne, Australia; Department of Neuropediatrics (C.H., H.M., S.V.S., I.H.), University Medical Center, Schleswig-Holstein, Christian-Albrechts University, Kiel, Germany; Danish Epilepsy Centre (R.S.M., H.H., M.N.), Daniafand, Institute for Regional Health Services (H.H., M.N.), University of Southern Denmark; Odense, Denmark; Department of Molecular and Medical Genetics (B.J.O.), Oregon Health and Science University, Portland, Oregon Institute (S.P., A.C., E.V.G., I.E.S.), Victoria; TY Nelson Department of Neurology (D.G.), The Children's Hospital at Westmead, Sydney, NSW, Australia; Department of Paediatrics (L.G.S.), School of Medicine and Health Sciences, University of Otago, Wellington, New Zealand, Epilepsy Research Program (B.L.H., I.M.D.), School of Pharmacy and Medical Sciences, University of South Australia, Adelaide; Division of Neurology (P.D.J.), Antwerp University Hospital, Belgium; and the Department of Paediatrics (I.E.S.), University of Melbourne, Royal Children's Hospital, Australia.

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Patients: WES cohort. Probands 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 (hemiconic 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 10 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, removing all potential PCR duplicates. The Genome Analysis Toolkit 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 (STXBP1, GABRA1, SCN1B, ATP12VOC, SLC8A1, CLSNT1, NKAIN3, NOL11, RIMS2, KIF1B, CDK5RAP3, ABT22, 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 (STXBP1, GABRA1, SCN1B) were previously implicated in epileptic encephalopathies or other epilepsies. An additional 5 genes (ATP12VOC, 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, ABT22, 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.

GABA4 mutagenesis and in vitro transcription. Human GABA4 complementary DNA (cDNA) was cloned into the pGEMHE vector containing a T7 promoter for in vitro transcription. The Gly251Ser mutation was generated using QuickChange Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA) with primers forward 5′-GAAGAGAAAGATAGCTACTTTGGTTATTCAACATACCTGGCC and reverse 5′-GGCGAGGATGTGTTGATGAAAGATAGCTACTTTGGTTATTCAACATACCTGGCC. transfection. Oocytes were examined using the Roboocyte Robot (Multi Channel Systems, Reutlingen, Germany) and scored for 2 days prior to experimentation. Two-electrode voltage clamp recordings were made in 96-well plates using the Roboocyte automated platform. Oocytes were loaded with 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 CaCl2 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 detected.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y/sex</th>
<th>Seizure onset age (mo), seizure type</th>
<th>Seizure types</th>
<th>Seizure sensitivity</th>
<th>Intellect (regression)</th>
<th>EEG</th>
<th>MRI</th>
<th>Likely causative gene</th>
<th>Testing method</th>
</tr>
</thead>
<tbody>
<tr>
<td>T20744</td>
<td>2/F</td>
<td>8, Brief H</td>
<td>FDS, H, SE, TCS</td>
<td>Present</td>
<td>Mild delay (no)</td>
<td>Normal</td>
<td>Normal</td>
<td>GABRA1</td>
<td>WES</td>
</tr>
<tr>
<td>T16706</td>
<td>7/F</td>
<td>11, Febrile, 20 min TCS</td>
<td>Ab, FDS, H, Myo, TCS</td>
<td>Present</td>
<td>Moderate ID (yes)</td>
<td>GSW</td>
<td>Normal</td>
<td>GABRA1</td>
<td>Targeted resequencing</td>
</tr>
<tr>
<td>T23532</td>
<td>18/M</td>
<td>11, Febrile, 10 min H</td>
<td>Ab, At, FDS, H, SE, T, TCS</td>
<td>Present</td>
<td>Moderate ID (yes)</td>
<td>Focal discharges</td>
<td>Calcified subependymal nodule in left lateral ventricle</td>
<td>GABRA1</td>
<td>Targeted resequencing</td>
</tr>
<tr>
<td>Co05</td>
<td>16/M</td>
<td>8, H SE</td>
<td>Ab, At, FDS, H, Myo, SE, TCS</td>
<td>Present</td>
<td>Mild ID (unknown)</td>
<td>GSW, MFD, PPR</td>
<td>Normal</td>
<td>GABRA1</td>
<td>Targeted resequencing</td>
</tr>
<tr>
<td>T1915</td>
<td>11/M</td>
<td>11, Afebrile cluster of TCS</td>
<td>At, FDS, Myo, SE, T, TCS</td>
<td>Present</td>
<td>Severe ID, deceased aged 11 y (yes)</td>
<td>MFD</td>
<td>Normal</td>
<td>STXBP1</td>
<td>WES</td>
</tr>
<tr>
<td>EP1807</td>
<td>21/M</td>
<td>6, Febrile FDS</td>
<td>Ab, FDS, Myo, TCS</td>
<td>Present</td>
<td>Severe ID (yes)</td>
<td>MFD</td>
<td>Atrophy</td>
<td>STXBP1</td>
<td>Targeted resequencing</td>
</tr>
<tr>
<td>T21717</td>
<td>6/F</td>
<td>12, Brief febrile TCS</td>
<td>Ab, At, H, Myo, T, TCS</td>
<td>Present</td>
<td>Learning difficulties (no)</td>
<td>Normal</td>
<td>ND</td>
<td>STXBP1</td>
<td>Targeted resequencing</td>
</tr>
<tr>
<td>T888</td>
<td>23/F</td>
<td>6, Afebrile H SE</td>
<td>Ab, FDS, H, Myo, SE, TCS</td>
<td>Present</td>
<td>Moderate ID (no)</td>
<td>GSW, PPR</td>
<td>Normal</td>
<td>SCN1A</td>
<td>WES</td>
</tr>
<tr>
<td>T1895</td>
<td>17/M</td>
<td>11, Febrile SE</td>
<td>aAb, At, FDS, Myo, NCS, SE, TCS</td>
<td>Present</td>
<td>Severe ID, deceased aged 20 y (yes)</td>
<td>GSW, PPR, MFD</td>
<td>Normal</td>
<td>SCN1A</td>
<td>WES</td>
</tr>
<tr>
<td>T17775</td>
<td>7/F</td>
<td>3, 10 min afebrile TCS</td>
<td>Ab, At, FDS, H, Myo, NCS, SE, TCS</td>
<td>Present</td>
<td>Severe ID (yes)</td>
<td>GSW, PSW, MFD</td>
<td>Normal</td>
<td>SCN1A</td>
<td>WES</td>
</tr>
<tr>
<td>T22809</td>
<td>3/M</td>
<td>6, Febrile SE</td>
<td>Ab, Myo, SE, TCS</td>
<td>Present</td>
<td>Mild ID (yes)</td>
<td>Normal</td>
<td>Normal</td>
<td>SCN1B</td>
<td>WES</td>
</tr>
<tr>
<td>T20038</td>
<td>10/F</td>
<td>6, Brief febrile TCS</td>
<td>TCS</td>
<td>Present</td>
<td>Mild ID (no)</td>
<td>Normal</td>
<td>Normal</td>
<td>None</td>
<td>WES</td>
</tr>
<tr>
<td>T16660</td>
<td>26/M</td>
<td>2, Brief TCS</td>
<td>Ab, At, FDS, Myo, SE, TCS</td>
<td>Present</td>
<td>Mild ID (unknown)</td>
<td>Focal discharges</td>
<td>Cerebellar atrophy</td>
<td>None</td>
<td>WES</td>
</tr>
<tr>
<td>T1911</td>
<td>8/M</td>
<td>7, TCS</td>
<td>At, FDS, Myo, T, TCS</td>
<td>Present</td>
<td>Severe ID (yes)</td>
<td>GSW, MFD</td>
<td>Normal</td>
<td>None</td>
<td>WES</td>
</tr>
<tr>
<td>T3892</td>
<td>9/M</td>
<td>4, Febrile SE</td>
<td>Myo, FDS, H, SE, TCS</td>
<td>Present</td>
<td>Moderate ID (no)</td>
<td>GSW, PSW, MFD</td>
<td>Normal</td>
<td>None</td>
<td>WES</td>
</tr>
<tr>
<td>T863</td>
<td>11/F</td>
<td>6, Ab</td>
<td>Ab, At, H, Myo, NCS, SE, T, TCS</td>
<td>Present</td>
<td>Mild ID (yes)</td>
<td>Normal</td>
<td>Delayed myelination</td>
<td>None</td>
<td>WES</td>
</tr>
<tr>
<td>T19264</td>
<td>9/F</td>
<td>14, Febrile TCS</td>
<td>Ab, FDS, H, Myo, SE, TCS</td>
<td>Present</td>
<td>Severe ID (no)</td>
<td>GSW, PPR</td>
<td>Normal</td>
<td>None</td>
<td>WES</td>
</tr>
<tr>
<td>T2985</td>
<td>39/M</td>
<td>6, Febrile, 15 min H</td>
<td>Ab, FDS, H, Myo, SE, TCS</td>
<td>Present</td>
<td>Moderate ID (no)</td>
<td>GSW, MFD</td>
<td>Normal</td>
<td>None</td>
<td>WES</td>
</tr>
</tbody>
</table>

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.

*Deceased.
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 (ATP6V0C, 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.

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

<table>
<thead>
<tr>
<th>Proband</th>
<th>Gene</th>
<th>Inheritance</th>
<th>cDNA change</th>
<th>GERP</th>
<th>Amino acid change</th>
<th>Polyphen score</th>
<th>ESP control allele frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T888</td>
<td>GAS2L2</td>
<td>De novo</td>
<td>c.2347G&gt;A</td>
<td>2.93</td>
<td>Arg783Trp</td>
<td>0.99</td>
<td>Not present</td>
</tr>
<tr>
<td>SCN1A</td>
<td>De novo</td>
<td>c.2044-1G&gt;A</td>
<td>5.47</td>
<td>Unk</td>
<td>NA</td>
<td>Not present</td>
<td></td>
</tr>
<tr>
<td>IGSF8</td>
<td>De novo</td>
<td>c.82G&gt;A</td>
<td>4.9</td>
<td>Arg27Trp</td>
<td>1</td>
<td>Not present</td>
<td></td>
</tr>
<tr>
<td>T17775</td>
<td>SCN1A</td>
<td>De novo</td>
<td>c.383C&gt;A</td>
<td>4.72</td>
<td>Ser128*</td>
<td>NA</td>
<td>Not present</td>
</tr>
<tr>
<td>T1895</td>
<td>SCN1A</td>
<td>De novo</td>
<td>c.1738G&gt;A</td>
<td>4.19</td>
<td>Arg580*</td>
<td>NA</td>
<td>Not present</td>
</tr>
<tr>
<td>T1915</td>
<td>STXBP1</td>
<td>De novo</td>
<td>c.847G&gt;A</td>
<td>5.32</td>
<td>Glu283Lys</td>
<td>0.97</td>
<td>Not present</td>
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<tr>
<td>T20744</td>
<td>GABRA1</td>
<td>De novo</td>
<td>c.751G&gt;A</td>
<td>5.05</td>
<td>Gly251Ser</td>
<td>1</td>
<td>Not present</td>
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<tr>
<td>T23532</td>
<td>GABRA1</td>
<td>De novo</td>
<td>c.335G&gt;A</td>
<td>5.85</td>
<td>Arg112Gln</td>
<td>0.83</td>
<td>Not present</td>
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<tr>
<td>T16706</td>
<td>GABRA1</td>
<td>Unk</td>
<td>c.335G&gt;A</td>
<td>5.85</td>
<td>Arg112Gln</td>
<td>0.83</td>
<td>Not present</td>
</tr>
<tr>
<td>Co05</td>
<td>GABRA1</td>
<td>De novo</td>
<td>c.917A&gt;C</td>
<td>5.47</td>
<td>Lys306Thr</td>
<td>0.99</td>
<td>Not present</td>
</tr>
<tr>
<td>T21717</td>
<td>STXBP1</td>
<td>De novo</td>
<td>c.853G&gt;T</td>
<td>5.95</td>
<td>Asp285Tyr</td>
<td>1</td>
<td>Not present</td>
</tr>
<tr>
<td>EP1807</td>
<td>STXBP1</td>
<td>De novo</td>
<td>c.1334A&gt;C</td>
<td>5.2</td>
<td>His445Pro</td>
<td>0.03</td>
<td>Not present</td>
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<tr>
<td>T22809</td>
<td>SCN1B</td>
<td>Maternal</td>
<td>c.363C&gt;G</td>
<td>2.49</td>
<td>Cys121Trp</td>
<td>1</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**Probands with mutations in known epilepsy genes**

- T1911: NKAIN3, De novo, c.2160>T, 5.53, Trp72Cys, 1, Not present
- ATP6V0C, De novo, c.1331_134delCT, 4.85, Ser45Cysfs*37, NA, Not present
- SLC8A1, De novo, c.2888G>C, 5.48, Cys963Ser, 1, Not present
- T16860: NOL11, De novo, c.144+1G>A, 4.86, Unk, NA, Not present
- T19264: FARP2, De novo, c.964C>G, 4.32, Leu322Val, 0.99, Not present
- COL6A3, De novo, c.8763_8764insT, -Proc2922Trfis*10
- T20038: CLSTN1, De novo, c.2607C>A, 4.71, Ser869Arg, 0.98, Not present
- CYP26C1, De novo, c.731A>G, 5.16, His244Arg, 0.42, Not present
- T2985p: 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

**Probands with mutations in candidate genes**

- T1911: NKAIN3, De novo, c.2160>T, 5.53, Trp72Cys, 1, Not present
- ATP6V0C, De novo, c.1331_134delCT, 4.85, Ser45Cysfs*37, NA, Not present
- SLC8A1, De novo, c.2888G>C, 5.48, Cys963Ser, 1, Not present
- T16860: NOL11, De novo, c.144+1G>A, 4.86, Unk, NA, Not present
- T19264: FARP2, De novo, c.964C>G, 4.32, Leu322Val, 0.99, Not present
- COL6A3, De novo, c.8763_8764insT, -Proc2922Trfis*10
- T20038: CLSTN1, De novo, c.2607C>A, 4.71, Ser869Arg, 0.98, Not present
- CYP26C1, De novo, c.731A>G, 5.16, His244Arg, 0.42, Not present
- T2985p: 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).

*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.

PProband was sequenced as mother-child pair; remaining probands were all sequenced as part of a proband-parent trio.

All variants were detected by targeted resequencing.

Parents unavailable to determine variant segregation.
with an allele frequency in the ESP dataset of <1% 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® 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.15,16

Autosomal recessive model for Dravet syndrome. Given that 2 recessive cases of Dravet syndrome have been reported,7,8 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, 1%) (table e-2). Targeted resequencing was performed in 7 candidate genes (RIMS2, KIF1B, CDR5RAP3, 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).7,8

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 *Xenopus 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 ± SEM: 2,621 ± 142, n = 97) compared to WT (max I ± SEM: 7,010 ± 325, n = 100) (figure 1B). Furthermore, the GABA dose-response curves showed a 5-fold decrease in
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 unexpected death in epilepsy. In →75% of cases, DS is associated with mutations of the gene encoding the sodium channel, SCN1A. However, the genetic causes of DS without mutations in SCN1A remain largely unknown.

Carvill et al.1 performed whole-exome sequencing in 13 SCN1A-negative DS candidates and targeted resequencing in 67 additional patient candidates to discover novel genes underlyng DS other than SCN1A. They identify 2 novel genes, GABRA1 and STXBP1, that have an association with DS; GABRA1 and STXBP1 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 γ-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.


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extracellular domain of the protein. p.Cys121Trp mutations induce a hyperexcitable state in vitro. 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. Another unaffected SCN1B c.363C>T (p.C121W) carriers (n = 6) have been reported, 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+. Recently, 2 reports of recessive SCN1B mutations causing Dravet syndrome have been published, although one had an atypical phenotype, but no heterozygous dominant mutations have been reported. 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 STXBP1. 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, STXBP1 mutations are seen in other epileptic encephalopathy phenotypes, suggesting considerable phenotypic heterogeneity compared to GABRA1.

GABRA1 and STXBP1 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. Sadler performed phenotypic analysis. Hiltrud Muhle performed phenotypic analysis. Sarah von Spiczak performed phenotypic analysis. Marina Nikanorova performed phenotypic analysis. Brie L. Hodgson performed experiments and data analysis. Elena V. Gauza performed experiments and data analysis. Avido Sul 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. Bekovic performed phenotypic analysis and critically reviewed the manuscript. Ingolf E. Scheffer supervised and designed the study, performed phenotypic analysis, and wrote the manuscript. Heather C. Melford supervised and designed the study and wrote the manuscript.

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DISCLOSURE
G. Carvill, S. Weckhuysen, J. McMahon, C. Harrmann, R. Møller, H. Hjalgrim, J. Cook, E. Geraghty, Brie 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. Sadler, H. Muhle, S. von Spiczak, M. Nikanorova, E. Gauza, and A. Sul 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, Arizona 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 Bioceodex. I. Helbig reports no disclosures relevant to the manuscript. S. Bekovic 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 GlassmillerKline, Athena Diagnostics, UCB, Bioceodex, and Janssen-Cilag EMEA; has received
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APPENDIX

Accession numbers

SCN1A (NM_001165963.1), STXBP1 (NM_003165.3), GABRA1 (NM_000866.5), SCN2A (NM_199037.3), ATRIP (NM_00198509.1), SLC6A1 (NM_021097.2), CTSN (NM_001009566.1), NKAIN1 (NM_173688.2), NOL11 (NM_015462), REM2 (NM_00100117.2), KIF1B (NM_051074.3), CDK5RAP3 (NM_0127897.1), ATRB2 (NM_145826.2), STX3 (NM_034144.4), KDM2B (NM_003296.4), STX13 (NM_00166271.1).

Web resources

BWA-v0.5.6 (http://bio-bwa.sourceforge.net/)
GATK-v2.2-9 (http://www.broadinstitute.org/gatk/)
BWA-v0.5.6 (http://bio-bwa.sourceforge.net/)
Online Mendelian Inheritance in Man (http://www.omim.org)
Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/)
Gorilla (http://cglab.princeton.edu/gorilla/)
Mutalyzer (https://mutalyzer.nl/index)
Allen brain atlas (http://www.brain-map.org/)
National Center for Biotechnology Information; 2012.

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GABA\textsubscript{A} and STXBP1: Novel genetic causes of Dravet syndrome

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