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# A mutation in *GABRB3* associated with Dravet syndrome

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Dravet syndrome is a rare and severe type of epilepsy in infants. Approximately, 70–80% of patients with Dravet syndrome have mutations in *SCN1A*, the gene encoding the alpha-1 subunit of the sodium channel, while some simplex patients have variants in one of several other genes, including but not limited to *GABRA1*, *SCN2A*, *STXBP1*, *GABRG2*, and *SCN1B*. In this study, we performed exome sequencing in six patients with *SCN1A*-negative Dravet syndrome to identify other genes related to this disorder. In one affected individual, we detected a novel de novo heterozygous missense variant, c.695G>A, p.(Arg232Gln), in *GABRB3*, the gene encoding the β3-subunit of the gamma-aminobutyric acid type A (GABAA) receptor, which mediates inhibitory signaling within the central nervous system. In summary, the data in this study identify *GABRB3* as a candidate gene for Dravet syndrome.

**KEYWORDS**
Dravet syndrome, exome sequencing, *GABRB3*, *SCN1A*

## 1 | INTRODUCTION

Dravet syndrome (DS), previously known as severe myoclonic epilepsy on infancy, is a rare and catastrophic type of epilepsy in infants with febrile or afebrile, generalized or unilateral, and clonic or tonic-clonic seizures that are often prolonged in children with previously normal development. The epilepsy is often refractory to standard antiepileptic medications, and the children often develop cognitive, behavioral, or motor impairments from their second year of life. The outcome of patients with DS is poor, with intellectual disability, and ongoing seizures in most patients (Dravet, 2011). The causes of DS include heterozygous variants in the sodium channel α1 subunit gene, *SCN1A*, in 70–80% of patients, with 90% of variants arising de novo (Claes et al., 2001; Parihar & Ganesh, 2013). In addition to *SCN1A*, variants in other genes such as *GABRG2*, *SCN2A*, and *SCN1B* have been reported in several DS patients (Kang & Macdonald, 2016; Ogiwara et al., 2012; Patino et al., 2009; Shi et al., 2009). Using exome sequencing (ES), variants of *GABRA1*, and *STXBP1* were recently discovered as

significant contributors to DS after *SCN1A* abnormalities had been excluded (Carvill et al., 2014). In the present study, we applied ES for six Vietnamese *SCN1A*-negative patients with DS to discover other genetic etiologies of this syndrome.

## 2 | SUBJECTS AND METHODS

The study subjects were six patients (five males and one female) that ranged in age from 2 to 10 years. The parents of four patients were also included in the study (the parents of the other two patients were not available for the study). The study was approved by the Institutional Review Board of Children Hospital 2 (Ref. No.: CS/N2/16/01HT). Written informed consent was obtained from the parents of all children involved in the study. The diagnostic criteria for DS included the following: normal early development; seizures beginning in the first year of life in the form of generalized or unilateral clonic seizures or myoclonic seizures; sensitivity to fever; a normal interictal EEG and a normal MRI at onset; and the presence of afebrile seizures. The diagnosis of DS was further confirmed with the emergence of other progressive symptoms, including slowing or arrest of cognitive

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development after 2 years of age, ataxic gait, pyramidal signs, persistence of clonic seizures, and continued sensitivity to fever (Dravet, 2011; Villeneuve et al., 2014). The clinical features of all patients included in this study are summarized in Table 1.

These patients had been previously screened for *SCN1A* point variants using Sanger sequencing. In addition, exonic deletion/duplications had been excluded using *SCN1A* multiplex ligation-dependent probe amplification. To confirm the biological relationship of the probands and their parents, parentage testing was performed using the PowerPlex® Fusion System (Promega, Madison, WI) in accordance with the manufacturer's instructions.

## 2.1 | Exome sequencing and variant validation

Genomic DNA was extracted from blood cells from the probands and their parents using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI) according to the manufacturer's instructions. Libraries were generated from the genomic DNA using the Sure-SelectXT Library Prep Kit (Agilent Technologies, Santa Clara, CA) and sequenced on an Illumina HiSeq 4000 (Illumina, San Diego, CA).

We used the Burrows Wheeler Aligner (Li & Durbin, 2009) to align short reads to the human reference genome (UCSC hg19). Both Platypus (Rimmer et al., 2014) and Genome Analysis Toolkit (GATK) (DePristo et al., 2011; McKenna et al., 2010) were used to call variants (i.e., SNPs and short indels) from four trios and two individual samples. The script bayesianDeNovoFilter.py developed together with Platypus was used to detect de novo variants from the four trios (Rimmer et al., 2014).

SNPeff software (Cingolani et al., 2012) was used to annotate the called variants. The minor allele frequencies of the variants were determined from three popular human variant databases: ExAC database (Lek et al., 2016), 1000 Genome Project database (1000 Genomes Project Consortium et al., 2015), and Exome Variant Server (NHLBI GO Exome Sequencing Project, 2016). The variants were also filtered for the presence of 11 known DS-related genes (*SCN1A*, *PCDH19*, *CHD2*, *GABRA1*, *GABRG2*, *STXBP1*, *SCN1B*, *SCN9A*, *SCN2A*, *SCN8A*, and *CACNB4*) and of 66 other known epileptic encephalopathy-related genes (*ALDH5A1*, *ALDH7A1*, *ALG13*, *ARX*, *ARHGEF9*, *CACNA1A*, *CDKL5*, *CHRNA2*, *CHRNA4*, *CHRNA7*, *CHRNB2*, *CYFIP1*, *DEPDC5*, *DNM1*, *EEF1A2*, *FOXG1*, *GABRB1*, *GABRB3*, *GNAO1*, *GRIN1*, *GRIN2A*, *GRIN2B*, *HCN1*, *HNRNPU*, *IQSEC2*, *KANSL1*, *KCNA2*, *KCNB1*, *KCNH1*, *KCNH5*, *KCNQ2*, *KCNQ3*, *KCNT1*, *LGI1*, *MBD5*, *MECP2*, *MEF2C*, *MTOR*, *NDE1*, *PIGA*, *PLCB1*, *PNKP*, *PNPO*, *POLG*, *PTEN*, *PURA*, *SIK1*, *SLC13A5*, *SLC1A2*, *SLC25A22*, *SLC2A1*, *SLC35A2*, *SLC6A1*, *SLC9A6*, *SPTAN1*, *STX1B*, *SYN1*, *SYNGAP1*, *TBC1D24*, *TCF4*, *TSC1*, *TSC2*, *UBE3A*, *WDR45*, *WWOX*, and *ZEB2*).

Sanger sequencing was performed to confirm candidate variants. Polymerase chain reaction (PCR) was used to amplify exon 9 of *SCN1A* with the primers forward: 5′- AGTACAGGGTGCTATGACCAAC and reverse: 3′- TCCTCATACAACCACCTGCTC (NG_011906) and exon 7 of *GABRB3* with the primers forward: 5′- CATTGCCTGTTGTTGACC-TATG and reverse: 3′- GGAATCGTGCGTGAAATTAGTG (NG_012836). Variant detection analysis was performed using CLC Main Workbench (QIAGEN Bioinformatics). The candidate variants

were interpreted and classified according to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines (Richards et al., 2015).

## 3 | RESULTS

The ES produced an average of ~5.9 GB of sequence per sample for 14 samples (four trios and two individual patients). The depth coverage of the samples ranged from 65× to 105×. Most of the short reads had high base quality, that is, ~96% of short reads with Phred-score quality ≥30.

We identified 22 de novo variants in the four trios, of which five were missense variants. The remaining 17 were synonymous changes, upstream gene variants, or intron variants in genes that have not been reported in epilepsy. Of five missense variants, one was a heterozygous variant in *GABRB3* (c.695G>A, p.[Arg232Gln]). This gene encodes the β3-subunit of the gamma-aminobutyric acid type A (GABAA) receptor, which mediates inhibitory signaling within the central nervous system. This missense variant in patient 3 (3, M) was detected by both Platypus and GATK methods with high confidence. The other four missense de novo variants were located in four other epilepsy-unrelated genes (i.e., *ALPK2*, *ZBED4*, *IFT140*, *STX16-NPEPL1*).

We also examined variants in the four trios called from Platypus and GATK methods in the panel of 11 DS-related genes and in the panel of 66 other epileptic encephalopathy-related genes. A variant was considered "good" and retained for further analyses if its Phred-score quality was ≥30 and its depth coverage was ≥4. Since, DS is a severe pediatric disease, variants with minor allele frequencies ≥0.01% were removed from the analyses. The number of good variants on a trio ranged from 196,000 to 343,000. In patient 1 (1, M), we detected one missense variant (i.e., c.1178G>A, p.[Arg393His]) in *SCN1A* that was not detected by our previous Sanger sequencing. Note that this variant was detected by both Platypus and GATK methods with high confidence. Interestingly, we also detected this alteration in the genome of the patient's father. The number of reads covering the variant site in the father of the patient was 49, of which 13 reads supported the variant. We hypothesize that this abnormal allele balance is due to somatic mosaicism.

For the two individual patients whose parents were not available for this study, the number of good variants for each individual patient was approximately 90,000. However, we did not identify any good variant with moderate or high impact among the 11 DS-related genes and 66 other epileptic encephalopathy-related genes in the two patients.

## 3.1 | Variant confirmation and evaluation

The two candidate variants identified by ES were confirmed by Sanger sequencing. For patient 1, the electropherograms confirmed the heterozygous variant c.1178G>A in *SCN1A* in the proband (supplementary Figure S1a), whereas the lower levels of the mutant allele supported the mosaicism in his father (supplementary Figure S1b). In

**TABLE 1** Summary of clinical characteristics of six patients

| Patient, gender | Age of last exam (y:m) | Seizure onset age (m), seizure type | Type of seizures | Fever sensitivity | EEG (onset; evolution) | MRI | Frequency of convulsive seizure and age of changing (y:m) | Total AED received | AED at last exam | AF (VVABS score) and other clinical features at last exam |
|---|---|---|---|---|---|---|---|---|---|---|
| 1, M | 1:9 | 4, Febrile SE | GTC, He, SE | YES | Normal, normal | Normal | (++) | VPA, CZP | VPA, CZP | Normal AF (81), hyperactivity |
| 2, M | 4:7 | 8, Brief febrile GTC | GTC, CP, At, My | YES | Normal; GSW (3y) | Normal | (++) | VPA, LTG, LEV | VPA, LEV | Moderate deficient AF (42), hyperactivity, ataxic gait |
| 3, M | 5:6 | 10, Brief febrile GTC | GTC, CP, My | YES | Normal; GSW, MFD (30 m) | Normal | (++) $\to_{3:10}$ (+) | VPA, OXC | (−) | Severe deficient AF (25), hyperactivity, autism |
| 4, M | 6:2 | 8, Brief afebrile GTC | GTC, My, CP, AA | YES | Normal; GSW, PSW, MFD (4y) | Atrophy | (++) $\to_{1:6}$ (+) $\to_{3:0}$ (++++) $\to_{5:6}$ (+++) | VPA, OXC, CZP, TPM, LEV | VPA, LEV, TPM | Severe deficient AF (24), hyperactivity, ataxic gait |
| 5, F | 8:8 | 8, Brief febrile CP | GTC, CP, He, SE, Ab | YES | Normal; GSW (5y) | Normal | (++) $\to_{1:1}$ (+++) $\to_{5:0}$ (+) | VPA, TPM, LEV, CBZ | LEV, CBZ | Profound deficient AF (19), hyperactivity, ataxic gait |
| 6, M | 8:11 | 11, Brief febrile GTC | CP, My, GTC, SE, At, AA | YES | Normal; GSW, MFD (3y) | Normal | (++) $\to_{2:0}$ (++++) $\to_{5:0}$ (+) | VPA, OXC, LTG, CBZ, PB, TPM | VPA, TPM | Profound deficient AF (14), hyperactivity, ataxic gait |

(+), <4 seizures/year; (++), 1–4 seizures/month; (+++), 5–10 seizures/month; (++++), >10 seizures/month; AED, antiepileptic drugs; At, atonic, AA, atypical absence; CBZ, carbamazepine; CLB, clobazam; CZP, clonozepam; CP, complex partial; EEG, electroencephalogram; F, female; GSW, generalized spike-waves; GTC, generalized tonic-clonic; He, hemiclonic; AF, adaptive functioning; LTG, lamotrigine; LEV, levetiracetam; My, myoclonic; MRI, magnetic resonance imaging; MFD, multifocal discharges; M, male; OXC, oxcarbazepine; PB, phenobarbital; PSW, polyspike waves; SE, status epilepticus; VPA, valporic acid; VGB, vigabatrin; y:m, year:month; VVABS, Vietnamese version of the Vineland Adaptive Behavior Scales.

patient 3, the altered allele appeared in the proband but not his parents, confirming the de novo heterozygous c.695G>A variant in *GABRB3* occurred in the patient (supplementary Figure S1c). The variant c.1178G>A in *SCN1A* is located in the pore-forming DIS5–S6 structure in Nav1.1, whereas the variant c.695G>A in *GABRB3* is located in the extracellular domain. Both variants, *SCN1A* (c.1178G>A) and *GABRB3* (c.695G>A), were absent from global human variant databases (i.e., ExAC database, 1000 Genomes Project database, and Exome Variant Server database). In silico analysis using all four annotation tools, Mutation Taster, Polyphen2, SIFT, and PROVEAN, predicted both variants to be disease causing, probably damaging, damaging, and deleterious, respectively. Using the guidelines developed by the American College of Medical Genetics and Genomics (ACMG) for the interpretation of sequence variants, both variants were classified as "pathogenic variants" (criteria PS1, PM1, PM2, PP2, and PP3 were met for the variant c.1178G>A in *SCN1A;* and criteria PS2, PM2, PM5, PP2, and PP3 were met for the variant c.695G>A in *GABRB3*).

## 4 | DISCUSSION

Exome sequencing in six patients with DS patients detected two variants in two patients that passed our filtering criteria: one variant in *GABRB3* and one in the *SCN1A* gene. Both are heterozygous missense variants. The variant in *GABRB3* is novel and occurred de novo, whereas the variant in *SCN1A* has been reported and was transmitted from the father, who appeared to be mosaic for the variant.

The gene *GABRB3* encodes gamma-aminobutyric acid type A receptor β3 and is a member of the gamma-aminobutyric acid type A ($GABA_A$) receptor gene family. This gene family encodes heteromeric pentameric ligand-gated chloride channels that act as the primary mediators of fast inhibitory synaptic transmission in the central nervous system. In humans, the $GABA_A$ receptor subunit genes include six types of α subunits (*GABRA1, GABRA2, GABRA3, GABRA4, GABRA5, GABRA6*), three βs (*GABRB1, GABRB2, GABRB3*), three γs (*GABRG1, GABRG2, GABRG3*), as well as a δ (*GABRD*), an ε (*GABRE*), a π (*GABRP*), and a θ (*GABRQ*). Most gamma-aminobutyric acid type A (GABAA) receptors have five subunits: two α, two β, and one other subunit (most frequently γ). When the GABAA receptor binds its physiological ligand, GABA, the ion pore opens, facilitating chloride influx or efflux (Macdonald, Kang, & Gallagher, 2012). Because of the central inhibitory role of GABAA receptors, any enhancement of the inhibitory actions of $GABA_A$ receptors could cause anti-convulsive effects, whereas a reduction in the function of these receptors could cause convulsions (Hirose, 2014; Macdonald et al., 2012). To date, variants in several GABAA receptor subunit genes including *GABRA1, GABRB3, GABRD*, and *GABRG2* have been found to be associated with several genetic epilepsy syndromes. In particular, variants in *GABRA1* and *GABRG2* had been reported in DS (Carvill et al., 2014; Huang, Tian, Hernandez, Hu, & Macdonald, 2012; Kanaumi et al., 2004).

Reduced gene expression of *GABRB3* has been postulated in the pathogenesis of Rett syndrome, Angelman syndrome, and autism spectrum disorders. These three neurological disorders share several clinical features, most notably neurodevelopmental delay and epilepsy (Hamdan et al., 2014; Urak, Feucht, Fathi, Hornik, & Fuchs, 2006). In addition, associations of single nucleotide polymorphisms and missense mutations in *GABRB3* and childhood absence epilepsy have previously been established (Tanaka et al., 2008; Urak et al., 2006). More recently, 13 cases of *GABRB3* epileptic encephalopathy were described with a total of 12 variants identified. These included five patients with infantile spasms and Lennox–Gastaut syndrome, one patient with epilepsy with myoclonic-atonic seizures (Doose syndrome), six patients with non-specific epileptic encephalopathy, and one patient with DS-like phenotype. (Epi4 K Consortium, 2016; Epi4 K Consortium et al., 2013; Hamdan et al., 2014; Papandreou et al., 2016). For the DS-like patient, the *GABRB3* variant segregated in a family with genetic epilepsy and febrile seizures plus (GEFS+). This patient had features reminiscent of DS but could be distinguished by the lack of some typical DS features, including generalized spike wave activity and hemiclonic and focal seizures. In the present study, the patient 3 with the *GABRB3* variant exhibited a more typical DS presentation. This patient was the first male child born to a nonconsanguineous couple and there was no family history of seizures. The pregnancy was normal, and the boy was born uneventfully in 2010. At 10 months of age, the boy had his first febrile seizure. He had no infantile spasms but generalized tonic-clonic seizures since onset and myoclonic seizures and complex partial seizures since the second year of life. In particular, his generalized tonic-clonic seizures were very sensitive to fever. EEG and MRI findings at onset were normal, but the interictal EEG at 30 months showed slowing of the background and multifocal sharp-waves. Generalized spike-waves were induced by photic stimulation, and there were no specific characteristics of other epileptic encephalopathy, including West syndrome, Lennox–Gastaut syndrome, or Doose syndrome. His early development during the first year of life was normal but was followed by severe developmental delay, with an adaptive functioning score at 5 years and 6 months of 25, as assessed by the Vietnamese version of the Vineland Adaptive Behavior Scales. He was also very hyperactive and was diagnosed with autism.

The *GABRB3* protein consists of a large extracellular NH2-terminal region, four hydrophobic transmembrane domains (M1-M4), an M3–M4 intracellular loop, and a short, barely extruding COOH-terminus (Vithlani, Terunuma, & Moss, 2011). Of the four transmembrane segments, the M2 segment lines the ion channel pore with the other four subunits. The de novo heterozygous variant, c.695G>A (p. [Arg232Gln]) is located in the extracellular domain (at amino acid 232), near the M1 segment (Figure 1). Among 12 variants previously associated with epileptic encephalopathies, six were also located in the extracellular NH2-terminal region (at amino acid positions 110, 120, 138, 157, 180, and 182), five were located in hydrophobic transmembrane domains (at amino acid positions 249, 256 [in the
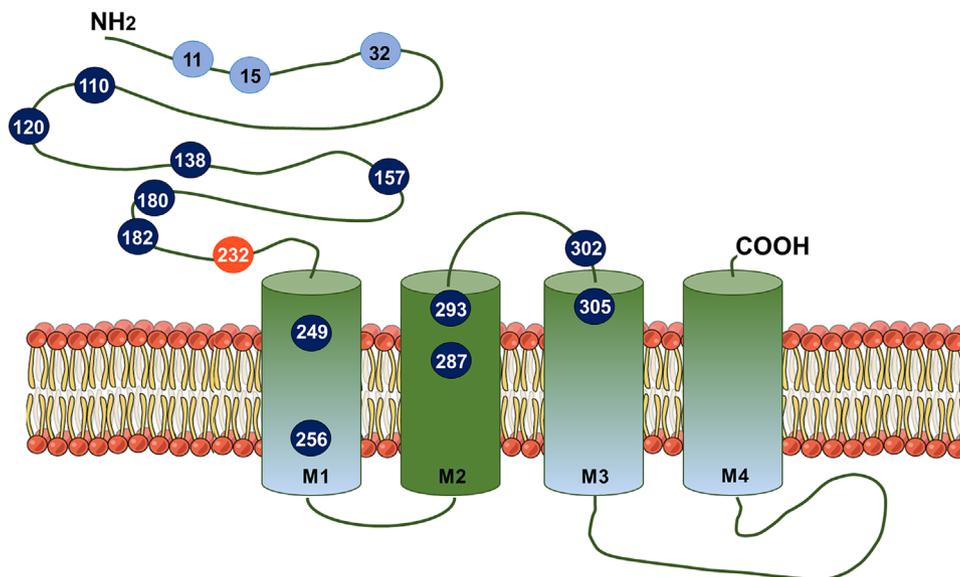
**FIGURE 1** The gamma-aminobutyric acid type A receptor β3 (GABRB3) subunits, including the extracellular domain, M1-M4 transmembrane bundle, and M3-M4 intracellular loop. The de novo heterozygous variant in GABRB3 in patient 3 of the present study was located at amino acid position 232, which is located in the extracellular domain. Previously reported variants of GABRB3 in patients with epileptic encephalopathies were located at amino acid positions 110, 120, 138, 157, 180, 182, 249, 256, 287, 293, 302, and 305. Related variants in childhood absence epilepsy have been detected at positions 11, 15, and 32, closer to the N terminus. Color figure can be viewed at wileyonlinelibrary.com

M1 segment], 287, 293 [in the M2 segment], and 305 [in the M3 segment]), and one in the M2-M3 extracellular loop (at amino acid position 302) (Epi4 K Consortium, 2016; Epi4 K Consortium et al., 2013; Hamdan et al., 2014; Papandreou et al., 2016). On the other hand, polymorphisms and variants implicated in childhood absence epilepsy were also found in the extracellular NH2-terminal region but clustered closer to the N terminus (at amino acid positions 11, 15, and 32) (Hirose, 2014; Lachance-Touchette et al., 2010; Tanaka et al., 2008) (Figure 1).

Furthermore, a search of Clinvar identified another amino acid change at the same position that we detected (p.[Arg232Pro]). This variant, submitted by Baylor Miraca Genetics Laboratories, was found via clinical testing in a 20-year-old female with childhood absence and was classified as "likely pathogenic." Considering the large difference in physicochemical properties between proline and glutamine as well as the possible involvement of other genetic and environmental factors, the distinct phenotypes in this female patient and patient 3 of the present study are not surprising. It is interesting, however, to note that this female also had intellectual disability, an important feature of epileptic encephalopathy.

In conclusion, this study is the first report of a variant in *GABRB3* associated with a typical DS phenotype.

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