

# Rare Copy Number Variants Are an Important Cause of Epileptic Encephalopathies

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**Objective:** Rare copy number variants (CNVs)—deletions and duplications—have recently been established as important risk factors for both generalized and focal epilepsies. A systematic assessment of the role of CNVs in epileptic encephalopathies, the most devastating and often etiologically obscure group of epilepsies, has not been performed.

**Methods:** We evaluated 315 patients with epileptic encephalopathies characterized by epilepsy and progressive cognitive impairment for rare CNVs using a high-density, exon-focused, whole-genome oligonucleotide array.

**Results:** We found that 25 of 315 (7.9%) of our patients carried rare CNVs that may contribute to their phenotype, with at least one-half being clearly or likely pathogenic. We identified 2 patients with overlapping deletions at 7q21 and 2 patients with identical duplications of 16p11.2. In our cohort, large deletions were enriched in affected individuals compared to controls, and 4 patients harbored 2 rare CNVs. We screened 2 novel candidate genes found within the rare CNVs in our cohort but found no mutations in our patients with epileptic encephalopathies. We highlight several additional novel candidate genes located in CNV regions.

**Interpretation:** Our data highlight the significance of rare CNVs in the epileptic encephalopathies, and we suggest that CNV analysis should be considered in the genetic evaluation of these patients. Our findings also highlight novel candidate genes for further study.

ANN NEUROL 2011;70:974–985

Epilepsy is often associated with major comorbidities, static or progressive. In the former, a variety of disorders most frequently cognitive difficulties, which can be are believed to cause both static intellectual disability and

View this article online at [wileyonlinelibrary.com](http://wileyonlinelibrary.com). DOI: 10.1002/ana.22645

Received May 21, 2011, and in revised form Aug 18, 2011. Accepted for publication Aug 26, 2011.

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epilepsy; in the latter, the epileptic process contributes to cognitive impairment. Epileptic encephalopathies (EEs), recently defined as severe epilepsies in which the epileptic activity, in addition to the seizures, contributes to cognitive impairment or regression, account for a significant proportion of the refractory epilepsies usually associated with poor outcome.<sup>1</sup> Most of the EEs begin in infancy or childhood, often in the setting of normal development with subsequent cognitive decline. In this way, the EEs differ considerably from disorders with static intellectual disability.

Copy number variants (CNVs) have been established as an important source of mutation in many neurocognitive and neuropsychiatric conditions, including intellectual disability (ID), autism spectrum disorders, and schizophrenia.<sup>2</sup> CNVs are regarded as causative in >10% of cases of ID.<sup>3</sup> More recently, both targeted<sup>4-6</sup> and genome-wide<sup>7,8</sup> discovery of CNVs in individuals with epilepsy have also established the importance of CNVs in the epilepsies. We previously evaluated >500 individuals with various types of pharmaco-responsive epilepsy for rare, potentially pathogenic CNVs, which were present in nearly 10% of patients.<sup>7</sup> The majority of patients in that series had genetic (idiopathic) generalized or focal epilepsies. However, a small number had more severe forms of epilepsy, and a greater percentage of these patients carried rare, potentially pathogenic CNVs. Although there have been reports of CNVs in some cases,<sup>9-11</sup> the EEs have not been systematically interrogated for CNVs as a group.

In this study, we hypothesized that some EEs could be caused by CNVs and that genes within those CNVs would be novel candidate genes for EEs. We selected a cohort of 315 patients with EEs. We performed high-density, whole-genome array comparative genomic hybridization (CGH) to determine what proportion of severe epilepsies may be caused by rare CNVs and to identify novel candidate genes within those regions.

## Patients and Methods

### Patient Samples

Patients with EEs for whom no cause was known were ascertained by referral and from the investigators' clinical practices. Detailed epilepsy, developmental, and general medical history was obtained with electroencephalography (EEG) and neuroimaging results. A seizure questionnaire was completed with the parents or caregivers where possible.<sup>12</sup> Strenuous efforts were made to obtain all previous medical records. Data were analyzed to determine each patient's phenotype and epilepsy syndrome according to the International League Against Epilepsy (ILAE) classifications.<sup>1,13-15</sup> Patients who did not have a phenotype consistent with a well known epilepsy syndrome, but

who did have an encephalopathic EEG with high-voltage diffuse background slowing and frequent epileptiform activity, and developmental slowing or regression, were classified according to the epileptiform pattern. For example, those with generalized spike and slow wave or sharp and slow wave activity were called symptomatic generalized epilepsies (SGEs) and those with unifocal or multifocal epileptiform abnormalities were classified as focal epilepsies with regression.

This study was carried out with approval from the Human Research Ethics Committees of Austin Health and the Royal Children's Hospital (Victoria, Australia) and the human subjects review board at the University of Washington (Seattle, WA). All subjects or, in the case of minors or individuals with intellectual disability, parents or legal guardians gave informed consent to participate.

### Array CGH and Analysis

We performed oligonucleotide array CGH using commercially available whole-genome, exon-focused arrays with 720,000 isothermal probes (Human CGH 3x720K Whole-Genome Exon-Focused Array, Roche NimbleGen, Madison, WI). Probes were preferentially placed in exonic sequences but also distributed throughout nonexonic regions. Probe spacing was variable with a mean spacing of ~4.2kb. Data were analyzed using Nimble Scan software followed by a 3-state hidden Markov model as previously described.<sup>7</sup> CNV calls were then filtered to eliminate: (1) events comprising <5 probes; (2) events that did not overlap any RefSeq genes; (3) events entirely within segmental duplications; and (4) events with >50% overlap with a CNV detected in 4,519 published controls, taking into account probe coverage and ability to detect a given CNV in those controls.<sup>16,17</sup> For a subset of genomic regions that are prone to recurrent rearrangement and known to be associated with a range of neurocognitive disorders, we did not require absence of CNVs in controls, as it is well established that there is incomplete penetrance and a small number of controls carry CNVs in some of these regions (eg, 15q13, 16p11.2, 16p13, and 15q11.2; see Hotspot CNVs). All filtered events were also visually inspected in a genome browser. Candidate rare CNVs not seen in controls (all CNVs listed in Table 2) were validated using custom high-density arrays (Agilent Technologies, Santa Clara, CA). Parents were analyzed, where available, to determine if the CNV had arisen de novo or was inherited; parental phenotypes were taken into account to help to determine if the CNV was significant. We also considered CNV size and gene content when evaluating the likely pathogenicity of a CNV, following guidelines that have been published for the interpretation of clinical array CGH results in patients with ID, autism, or multiple anomalies.<sup>3</sup> Deletions involving known epilepsy genes (*CDKL5*, *UBE3A*, and *CNTNAP2*) were considered pathogenic. De novo deletions were considered pathogenic; de novo duplications were considered likely pathogenic; CNVs >1Mb that were inherited or of unknown inheritance were deemed likely pathogenic. In 2 cases, an inherited 500kb duplication of 16p11.2 was considered likely pathogenic because of the known disease associations with duplications at this locus as it may be

**TABLE 1: Phenotypes of Patients Included in This Study**

Diagnosis	n	With Rare CNV(s), n (%)
MAE	77	6 (7.8)
SGE	65	7 (10.8)
Infantile spasms	44	3 (6.8)
Epilepsy-aphasia syndrome/CSWS	29	3 (10.3)
Lennox-Gastaut syndrome	20	0 (0.0)
Dravet syndrome <sup>a</sup>	19	1 (5.3)
MPSI	11	1 (9.1)
DESC/FIRES	10	0 (0.0)
FE with regression <sup>b</sup>	17	4 (23.5)
Other <sup>c</sup>	23	0 (0.0)
Total	315	25 (7.9)

<sup>a</sup>All Dravet patients have been screened for SCN1A mutations by sequence and MLPA.

<sup>b</sup>FE with regression includes severe infantile multifocal epilepsy (n = 2), multifocal epileptic encephalopathy and unifocal epilepsy with regression.

<sup>c</sup>Other includes progressive myoclonic epilepsy (n = 6), atypical benign partial epilepsy (n = 1), Ohtahara syndrome (n = 2), other (n = 14).

CNV = copy number variant; CSWS = continuous spike and wave during slow sleep; DESC = devastating epileptic encephalopathy in school-age children; FE = focal epilepsy; FIRES = febrile infection-related epilepsy syndrome; MAE = epilepsy with myoclonic atonic seizures; MLPA = multiplex ligation-dependent probe amplification; MPSI = migrating partial seizures of infancy; SGE = symptomatic generalized epilepsy.

contributing to the epilepsy phenotype. All other CNVs were considered of unknown significance due to size (<500kb), inheritance (inherited or unknown), or gene content (no known epilepsy genes).

### Sequence Analysis

For each candidate gene (*CACNA2D1* and *LRRK2*), targeted sequencing was performed for all exons, exon-intron boundaries, and 2kb upstream of the transcription start site using standard Sanger sequencing. Primer sequences are available upon request. *CACNA2D1* was resequenced in 94 probands including 80 cases with epilepsy with myoclonic atonic seizures (MAE), 13 with Dravet syndrome, and 1 with genetic epilepsy with febrile seizures plus (GEFS+) phenotype. Of these, 92 were from our cohort of 315 cases; 2 additional cases were selected for resequencing only. *LRRK2* was resequenced in all 11 cases from our cohort with a diagnosis of migrating partial seizures of infancy (MPSI).

## Results

We studied a cohort of 315 unrelated patients with epileptic encephalopathy (EE; Table 1). We identified 25 of 315 (7.9%) patients with 1 or more rare CNVs not seen in controls (Table 2). The mean CNV size was 2.26Mb and median size was 510kb. Of these, 13 patients (4.1%) had CNVs that were clearly pathogenic (n = 8) or likely pathogenic (n = 5), and 12 had 1 or more rare CNVs that were not seen in controls but were of unclear clinical significance. Four individuals had 2 rare CNVs.

### Inheritance

We evaluated 1 or both parents in all 25 cases to determine the inheritance pattern of all 29 rare CNVs (see Table 2). We identified 9 de novo, 8 maternally inherited, and 7 paternally inherited CNVs. In 1 case (T2761), the proband had a homozygous deletion and both parents were confirmed to be heterozygous carriers. In 3 cases (4 CNVs total) the CNVs were not present in the mother, but the father was unavailable for analysis.

### Pathogenic CNVs

In 13 patients we considered the CNV(s) to be pathogenic or likely pathogenic based on size, gene content, de novo inheritance, or the previous literature (see Table 2). Clearly pathogenic CNVs include heterozygous deletions that disrupt a single, known gene in 2 cases: *UBE3A* in case T3334 and *CDKL5* in case T2959. We also identified a homozygous deletion removing exon 2 of *CNTNAP2* in case T2761 and his affected sibling. Ten other cases have CNVs encompassing multiple genes. Two probands have overlapping deletions of 7q21: T964 with a de novo 8Mb deletion of 7q11–q21 and T438 with a 4Mb deletion of 7q21 (Fig 1). The 7q21 deletions have a 3Mb region of overlap that includes 6 genes: *GNAI1*, *GNAT3*, *CD36*, *SEMA3C*, *HGF*, and *CACNA2D1*. Other pathogenic CNVs include deletions of 10p13 (T257), 5q33–q34 (8893), and 4p16 (T1962) as well as 1 case with deletion of 9p24–p23 and adjacent duplication of 9p23–p21 (T3729). Likely pathogenic CNVs include a duplication of 16p11.2 in 2 cases (Fig 2) and a deletion of 1q44 (T2363). In addition, we identified a likely pathogenic, novel, de novo 700kb duplication of 1q32 in case T3810 (Fig 3). Additional rare CNVs of uncertain significance were present in 12 patients (see Table 2, Fig 3).

### Hotspot CNVs

Certain regions of the genome are “hotspots” for recurrent CNVs due to the presence of large blocks of duplicated DNA that facilitate non-allelic homologous recombination at meiosis.<sup>18</sup> We identified 5 individuals with

**TABLE 2: Rare Copy Number Variants Identified in a Cohort of 315 Individuals Affected by Epileptic Encephalopathy**

Proband	Chromosome	Build 36 Coord (Mb)	Size	CNV	Inheritance	Syndrome	Seizure Type(s) <sup>a</sup> and Intellect	Causal <sup>b</sup> Gene(s)
T2363	1q44	chr1: 242.62–244.06	1.44Mb	del	Not in mother	SGE	<u>FS</u> , <u>GTCS</u> , <u>M</u> , <u>CSE</u> , <u>T</u> ; <u>L</u> severe ID	<i>HNRNPU</i> , <i>EFCAB2</i>
T2761	7q35	chr7: 146.09–146.15	60kb	del <sup>H</sup>	Parents heterozygous	FE with regression	<u>FDS</u> , <u>CSE</u> ; moderate ID	<i>CNTNAP2</i> <sup>e</sup>
T2959	Xp22	chrX: 18.21–18.50	290kb	del	de novo	IS	<u>T</u> , <u>Sp</u> , <u>M</u> , severe ID	<i>CDKL5</i>
T3334	15q11	chr15: 23.21–23.48	270kb	del	de novo <sup>c</sup>	MAE	<u>At</u> , <u>M</u> , <u>MSE</u> ; severe ID	<i>UBE3A</i>
T438	7q21	chr7: 79.06–82.95	3.9Mb	del	Inherited (M) <sup>d</sup>	MAE	<u>FS</u> , <u>At</u> <u>DA</u> , <u>Abs</u> , <u>GTCS</u> ; <u>L</u> borderline intellect	<i>CACNA2D1</i> , <i>PCLO</i>
T964	7q11–q21	chr7: 73.78–81.98	8.2Mb	del	de novo	SGE	<u>FS</u> , <u>GTCS</u> , <u>M</u> , <u>Atyp</u> <u>Abs</u> ; <u>P</u> severe ID	<i>MAGI2</i> , <i>CACNA2D1</i>
7257	10p13	chr10: 12.25–17.5	5.25Mb	del	de novo	MAE	<u>GTCS</u> , <u>Abs</u> , <u>M</u> , <u>At</u> <u>DA</u> , <u>P</u> <u>T</u> ; mild ID	
8893	5q33–q34	chr5: 156.18–162.63	6.45Mb	del	de novo	MAE	<u>FS</u> , <u>GTCS</u> , <u>M</u> , <u>Abs</u> ; mild ID	<i>GABRA1</i> , <i>GABRG2</i>
T1962	4p16	chr4: prer-1.50	1.5 Mb	del	de novo	EAS	<u>FS</u> , <u>GTCS</u> , <u>FDS</u> ; moderate ID	<i>SLC26A1</i>
T3729	9p24–p21	chr9: 1–9.22	9.22Mb	del	de novo	SGE	<u>T</u> , <u>GTCS</u> , <u>CSE</u> , <u>FDS</u> ; <u>P</u> moderate ID	
T16335	16p11.2	chr16: 29.5–30.0	500kb	dup	Inherited (P)	IS	<u>Sp</u> ; mild ID	<u>L</u>
T2547	16p11.2	chr16: 29.5–30.0	500kb	dup	Inherited (P)	MFEE	<u>GTCS</u> , <u>HC</u> , <u>SGS</u> , <u>FDS</u> , <u>L</u> <u>T</u> ; mild ID	
T3810	1q32	chr1: 200.72–201.40	700kb	dup	de novo	EAS	EEG only; mild ID	<u>L</u> <i>SYT2</i>
T2709	12q12	chr12: 38.86–39.05	190kb	del	Inherited (M)	MPSI	<u>FDS</u> , <u>MFS</u> , <u>Sp</u> , severe ID	<u>U</u> <i>LRKK2</i> <sup>e</sup>
	4q28	chr4: 130.14–130.21	70kb	del	Inherited (P)			<i>SCLT1</i> <sup>e</sup>
T3472	2q33	chr2: 206.79–207.03	510kb	del	Not in mother	DS	Febrile hemiclonic status, <u>U</u> <u>GTCS</u> , <u>HC</u> , <u>M</u> , <u>At</u> <u>DA</u> , <u>CSE</u> ; moderate ID	<i>GPR1</i> , <i>ZDBF2</i> , <i>ADAM23</i>

**TABLE 2 (Continued)**

Proband	Chromosome	Build 36 Coord (Mb)	Size	CNV	Inheritance	Syndrome	Seizure Type(s) <sup>a</sup> and Intellect	Causal <sup>b</sup>	Candidate Gene(s)
8245	15q13	chr15: 26.80–28.50	1.7Mb	del	Inherited (P)	MAE	<u>FS</u> , <b>GTCs</b> , At DA, Abs; normal intellect	U	
T1466	5p13	chr5: 37.4–38.0	600kb	del	Inherited (M)	SGE	<u>GTCs</u> , FS, T DA, <b>Abs</b> , Atyp Abs, T, At DA, M, NSE; severe ID	U	<i>GDNF</i>
T1456	6q23	chr6: 137.32–137.40	80kb	del	Inherited (P)	SGE	Cyanotic, Abs, M, T DA, <u>AT DA</u> , MSE; severe ID	U	<i>NHEG</i>
6184	Xq28	chrX: 148.49–148.55	60kb	del	Inherited (M)	SGE	<u>GTCs</u> , T DA, Abs, FDS, Atyp Abs, At, M, severe ID,	U	<i>TMEM185A<sup>c</sup></i>
T3467	20q13	chr20: 52.67–53.09	420kb	dup	Inherited (M)	CSWS	<b>FDS</b> , <b>SGS</b> ; mild ID	U	<i>DOK5<sup>c</sup></i>
	22q11	chr22: 18.62–20.29	1.67Mb	dup	Inherited (M)				
T892	3q11	chr3: 98.05–99.69	1.64Mb	dup	Inherited (M)	IS	<u>GTCs</u> , <b>Sp</b> , CSE, T, FDS; severe ID	U	<i>EPPHA6</i> , <i>GABRR3</i>
T18349	16p11.2	chr16: 28.7–29.0	300kb	del	Neither in mother	SGE	FS, <u>GTCs</u> , <b>Abs</b> , Atyp Abs, T, CSE, M; moderate ID	U	
	5p13	chr5: 36.55–36.85	300kb	del					<i>SLC1A3<sup>c</sup></i>
T19083	14q22	chr14: 50.23–50.37	1.35kb	del	Inherited (M)	MFEE	<b>FDS</b> , T DA, M; mild ID	U	<i>MIN<sup>c</sup></i>
T16681	22q11.21	chr22: 16.44–16.52	80kb	del	Inherited (P)	MFEE	FSD, aura, <b>SGS</b> , Ge, <u>GTCs</u> ; mild ID	U	<i>SLC25A18</i>
T18721	4q35	chr4: 184.18–185.23	1.05Mb	dup	Inherited (P)	MAE	<u>FS</u> , <u>GTCs</u> , <b>Abs</b> , Abs status, M, At DA; mild ID	U	

Additional clinical details can be found in Supplementary Table 1. Underline denotes presenting seizure type; bold seizure type denotes the predominant seizure type experienced by patient.

<sup>a</sup>Seizure types: At = atonic; Abs = absence; AbsEM = absences with eyelid myoclonias; Atyp abs = atypical absence; Cl = clonic; CSE = convulsive status epilepticus; DA = drop attack; FDS = focal dyscognitive seizure; Focal = focal seizures without impairment of consciousness; FS = febrile seizure; Ge = gelastic seizure; GTCs = generalized tonic-clonic seizure; HC = hemiclonic; M = myoclonic seizure; MFS = multifocal seizures; MSE = myoclonic status epilepticus; NSE = nonconvulsive status epilepticus; Sp = spasms; SGS = secondarily generalized seizures; T = tonic.

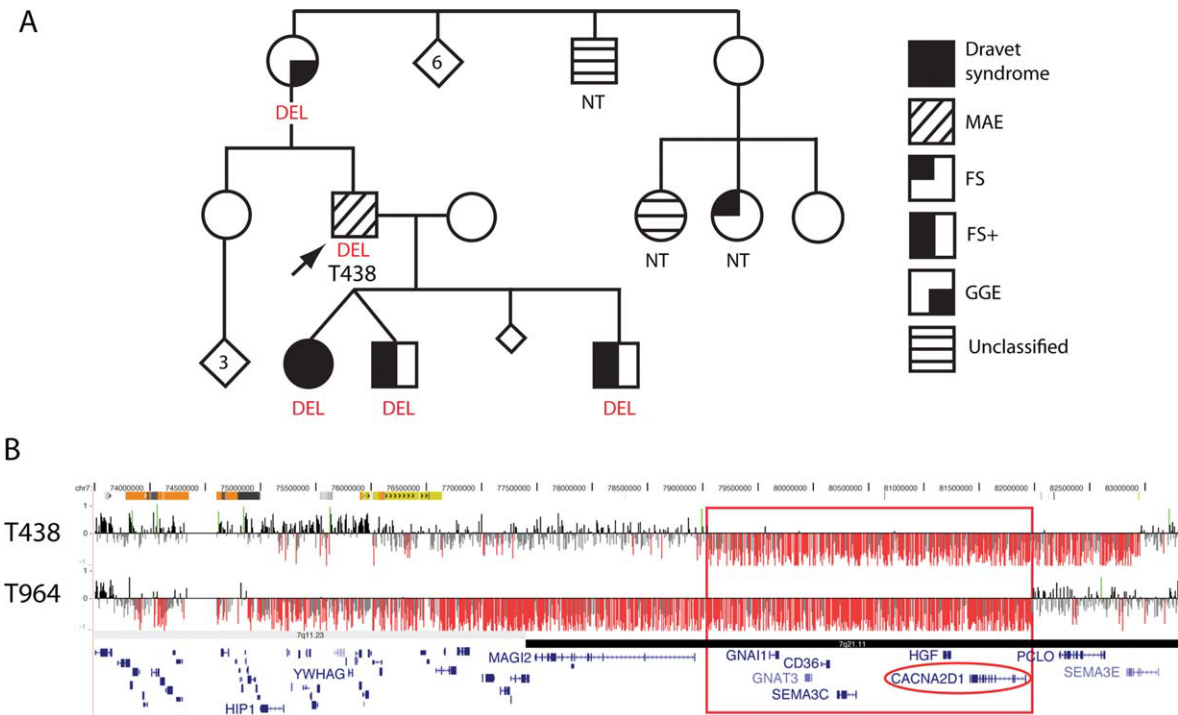
<sup>b</sup>P = pathogenic; L = likely pathogenic; U = uncertain.

<sup>c</sup>Maternal origin.

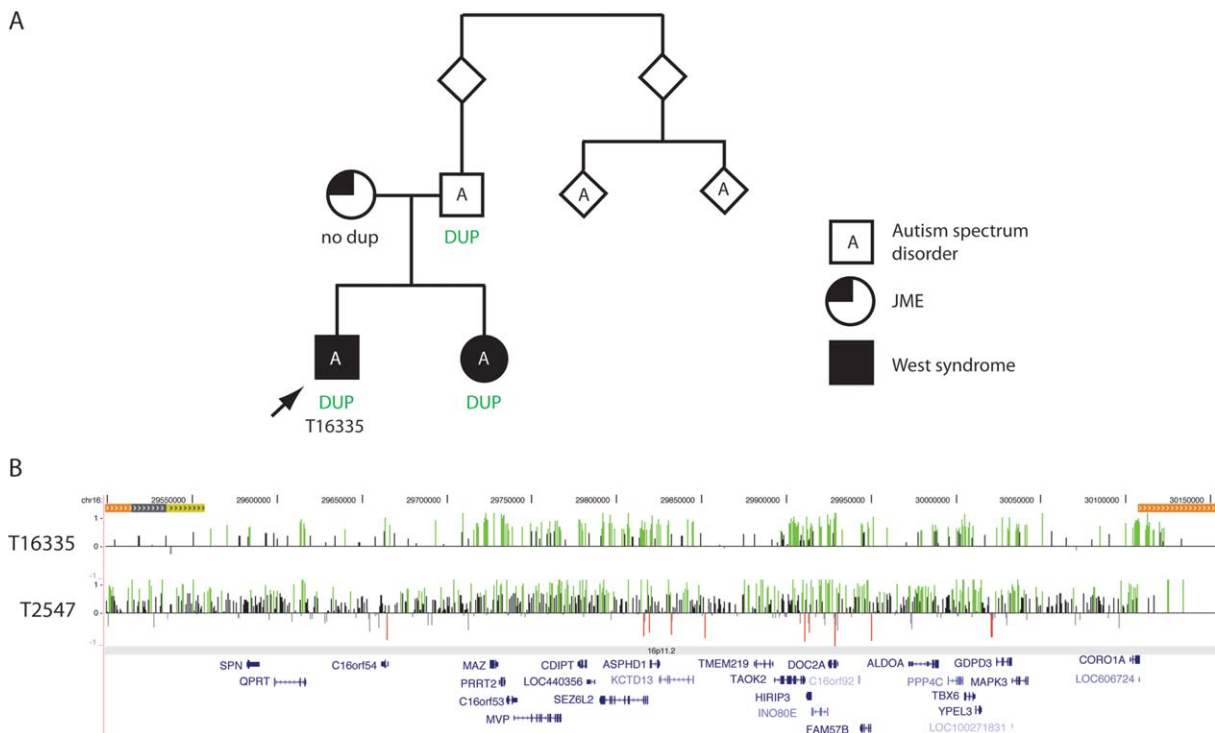
<sup>d</sup>Carrier parent also affected.

<sup>e</sup>Gene listed is the only gene affected by the CNV.

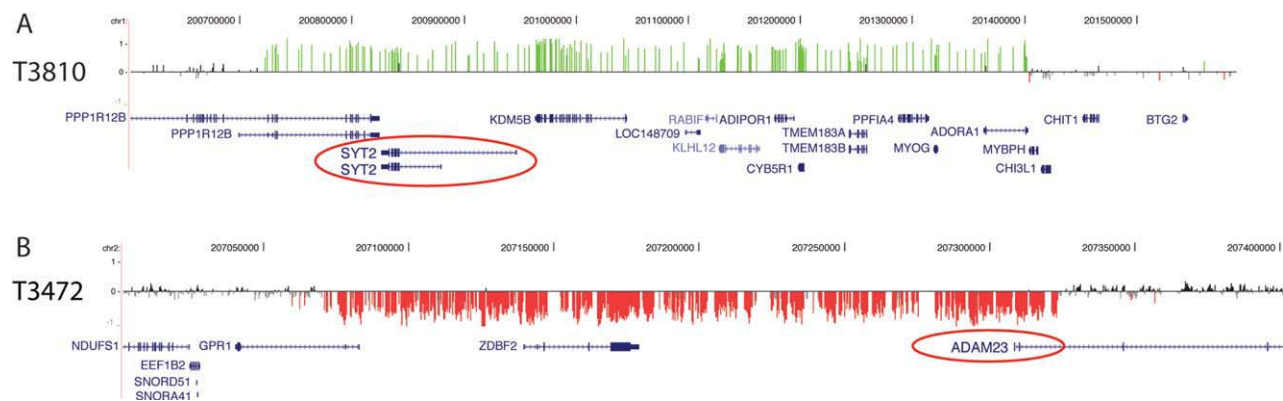
chr = chromosome; CNV = copy number variant; CSWS = continuous spike and wave during slow sleep; del = deletion; dup = duplication; del<sup>h</sup> = homozygous deletion; DS = Dravet syndrome; EAS = epilepsy-aphasia syndrome; FE = focal epilepsy; IS = infantile spasms; MAE = epilepsy with myoclonic atonic seizures; MFEE = multifocal epileptic encephalopathy; MPSI = migrating partial seizures of infancy; SGE = symptomatic generalized epilepsy.



**FIGURE 1: Overlapping deletions of 7q21 in 2 probands. (A)** Pedigree for proband T438, who has a ~4Mb deletion of 7q21. The proband’s mother and 3 children, who have all had 1 or more seizures, also have the same deletion. **(B)** Array CGH data for T438 and T964. The red box highlights the region on 7q21 that is deleted in both patients. X-axis represents genomic coordinates (chr7: 73.5–83.5Mb, NCBI Build 36). For each individual, deviations of probe log<sub>2</sub> ratios from zero are depicted by vertical gray/black lines, with those exceeding a threshold of 1.5 standard deviations from the mean probe ratio colored green and red to represent relative gains and losses, respectively. Genes are represented by blue lines at the bottom. MAE = epilepsy with myoclonic-atic seizures; FS = febrile seizures; FS+ = febrile seizures plus; GGE = genetic generalized epilepsies; DEL = deletion; NT = not tested.



**FIGURE 2: Recurrent duplications of 16p11.2 in 2 probands. (A)** Pedigree for proband T16335, who has a duplication of 16p11.2. The duplication is inherited from his father and also present in his sister with West syndrome. **(B)** Array CGH data for T16335 and T2547, with log<sub>2</sub> ratios displayed as in Figure 1. JME = juvenile myoclonic epilepsy; DUP = duplication.



**FIGURE 3:** Copy number changes affecting neuronal genes of interest. (A) 700kb de novo duplication of 1q32 in patient T3810. (B) 300kb deletion of 2p33 involving the *ADAM23* gene in T18349. Array CGH data displayed as in Figures 1 and 2.

CNVs at hotspot regions. Two individuals (T16335 with West syndrome and T2547 with a multifocal epileptic encephalopathy) have duplications of proximal 16p11.2 (chr16:29.5–30.0Mb; see Fig 2). We also identified rearrangements of uncertain significance at hotspot regions in case T18349 with SGE (distal 16p11.2 deletion), case T3467 with continuous spike and wave during slow sleep (CSWS; duplication of distal 22q11), and case 8245 with MAE (deletion of proximal 15q13.3, BP3–BP4) (see Table 2). We did not detect CNVs at distal 15q13.3 (BP4–BP5), 16p13.11, or 15q11.2, loci known to be important for GGE and some focal epilepsies.<sup>4,6,8</sup>

### Sequence Analysis of Candidate Genes

We selected 2 candidate genes for sequence analysis: *CACNA2D1* and *LRRK2* (Table 3). *CACNA2D1*, which is deleted in 2 patients (T438 with MAE and T964 with SGE), was resequenced in 94 probands. No convincing mutations were identified (see Table 3). *LRRK2*, deleted in patient T2709 with MPSI, was resequenced in 10 additional patients from our cohort with MPSI. In addition,

the nondeleted allele was sequenced in the original proband. No deleterious changes were identified.

### Discussion

We performed genome-wide exon-focused array CGH in a series of 315 patients with epileptic encephalopathies in order to identify novel genomic regions and candidate genes and to investigate how frequently CNVs were responsible for these phenotypes. Overall, we found that 7.9% of affected individuals carried at least 1 rare CNV. In 4.1%, we identified clearly pathogenic CNVs. These findings suggest that array CGH should be considered in the genetic evaluation of individuals with EE. Rare CNVs were most commonly identified in patients with focal epilepsy with regression, epilepsy-aphasia syndrome, and symptomatic generalized epilepsy (see Table 1) but were not identified in any of the patients with Lennox Gastaut syndrome or devastating epileptic encephalopathy in school-age children. Investigation of larger cohorts of specific EE should be carried out to further evaluate whether this apparent difference is significant.

**TABLE 3: CACNA2D1 Resequencing Results**

Gene	Patient	Sequence Change	AA Change	Phenotype	Inheritance
<i>CACNA2D1</i>	T1570	Exon 1, c.2382G>A <sup>a</sup>	S709N	MAE	Inherited from father
	9402	Exon 38, c.3390A>C <sup>b</sup>	D1045A	Mild MAE	Unaffected mother and dizygotic twin have same change
	T17741	Exon 38, c.3390A>C	D1045A	MAE	Unaffected father and affected monozygotic twin have same change

<sup>a</sup>Predicted to be benign by PolyPhen (<http://genetics.bwh.harvard.edu/pph>) and present in unaffected father.  
<sup>b</sup>Known rare polymorphism in European population and present in unaffected family members.  
 MAE = epilepsy with myoclonic atonic seizures.

Within our cohort, we identified both de novo and inherited CNVs. While we considered those that are de novo most likely to be pathogenic, many of the rare inherited CNVs contain brain-expressed genes involved in synaptic transmission and axonal guidance and are likely to contribute to the patient's phenotype. Indeed, even for the recurrent deletions at 15q13.3 and 16p13.11, which are clearly associated with epilepsy risk, there are many examples of unaffected carrier parents, incomplete penetrance and variable expressivity. The same may be true for some of the rare inherited CNVs identified in this study, and in these cases there may be additional genetic or non-genetic factors that also play a role in the clinical presentation.

### Pathogenic Events and Novel Candidate Genes

In several cases we found clearly pathogenic deletions that involved known epilepsy genes and had clear genotype-phenotype correlation, albeit atypical features in some instances. These include deletion of Xp22 resulting in the disruption of *CDKL5* in a boy who had an EE with the typical picture of a 3-stage evolution of seizures characteristically seen in girls with *CDKL5* encephalopathy<sup>19</sup> and also reported in rare male cases.<sup>10</sup> We also found an atypical deletion of 15q11 that disrupted *UBE3A* in a man with MAE who had features reminiscent of Angelman syndrome. Another man with MAE had a 6.5Mb deletion of 5q33–q34 encompassing a cluster of genes encoding subunits of the GABA-A receptor. Mutations in 2 of the genes in the deleted region, *GABRA1* and *GABRG2*, have been associated with JME<sup>20</sup> and GEFS+ and CAE,<sup>21,22</sup> respectively. We identified a homozygous deletion of exon 2 of the *CNTNAP2* gene in a pair of siblings of which the proband had focal epilepsy with regression; they shared some features in common with the Amish families reported by Strauss and colleagues.<sup>23</sup>

We also identified several CNVs that provide insight into potentially novel candidate genes for epilepsy. Two probands had overlapping deletions of 7q21. Deletions of 7q11–q21 distal to the Williams-Beuren syndrome locus have been associated with infantile spasms, with considerable interest in the gene *MAGI2*.<sup>24</sup> *YWHAG* and *HIP1* have also been proposed as candidate genes for the infantile spasm phenotype.<sup>25</sup> While patient T964 with SGE carried a large de novo deletion that included *MAGI2*, *YWHAG*, and *HIP1*, Patient T438 had a ~4Mb deletion that did not include these genes. Furthermore, neither of our patients had spasms. Given the similarity in phenotypes of the 2 probands, we focused on the 6 genes that were deleted in both patients as candidates for sequencing. One of the genes in the region of

overlap is *CACNA2D1*, which encodes the alpha-2/delta subunit of brain voltage-dependent calcium channels that bind the anti-epileptic drug, gabapentin.<sup>26</sup> Therefore, we considered *CACNA2D1* an excellent candidate gene for MAE and related phenotypes. We performed sequence analysis in 94 probands with MAE or Dravet syndrome phenotypes but did not detect any clearly deleterious mutations.

Interestingly, the smaller 4Mb deletion in the patient with MAE segregated with a range of epilepsy and cognitive phenotypes in the proband's family (see Fig 1). The deletion was found in family members with markedly different phenotypic severity ranging from the EEs of MAE and Dravet syndrome to febrile seizures plus, consistent with the GEFS+ spectrum, and even GGE.<sup>27</sup> All affected family members had learning difficulties; affected children all had marked behavioral problems. Thus the 7q21 deletion may be acting as 1 of several genetic factors that contribute to the more severe phenotype of MAE and Dravet syndrome in the setting of complex inheritance rather than acting as a monogenic cause. We have not screened *CACNA2D1* in other phenotypes, but given the presence of GGE in the proband's mother, a cohort of patients with GGE would be worth testing. Alternatively, another gene in the deleted region may be responsible for the phenotypes in this family.

We identified a 510kb deletion in patient T3472 with Dravet syndrome that disrupts the *ADAM23* gene (see Fig 3). The deletion is not found in the mother's genome, but we were unable to evaluate the father. *ADAM23* knockout mice have aberrant dendrite morphology and exhibit seizures in the neonatal period, and heterozygous mice are susceptible to PTZ-induced seizures.<sup>28</sup> Furthermore, *ADAM23* is part of a complex that contains *LGI1*, *ADAM22*, and *Kv1.1*<sup>29</sup> and has been shown to directly bind *LGI1*,<sup>28</sup> mutations of which cause autosomal dominant partial epilepsy with auditory features in humans.<sup>30,31</sup> It is possible that disruption of *ADAM23* in our patient leads to Dravet syndrome.

Case T3810 with an epilepsy-aphasia syndrome carries a de novo 700kb duplication of 1q32 that involves 13 genes (see Fig 3). One of these is *SYT2*, a member of the synaptotagmin family of genes that encode membrane proteins expressed at the synapse that are thought to act as calcium sensors.<sup>32,33</sup> It is possible that excess *SYT2* disrupts normal synaptic transmission in our patient.

### Hotspot CNVs

Recurrent deletions at three rearrangement "hotspots"—15q13.3 (BP4–BP5), 16p13.11, and 15q11.2—have recently been identified as important risk factors for



epilepsy.<sup>4–6,8</sup> Each of these deletions is found in up to 1% of individuals with epilepsy, and each has also been associated with ID, autism, and/or schizophrenia.<sup>34–41</sup> While heterozygous 15q13.3 deletions have been found almost exclusively in patients with GGE, deletions at the other loci have been found in patients with a broader range of epilepsies. We identified 1 patient with an inherited deletion BP3–BP4 on 15q13. This deletion is proximal to the BP4–BP5 deletion that has been associated with epilepsy, and the clinical significance is not clear.<sup>42</sup> Interestingly, we did not identify any CNVs at 15q13.3 (BP4–BP5), 16p13.11, or 15q11.2, suggesting that heterozygous deletions of these regions may be primarily associated with the more common and milder forms of epilepsy, although homozygous 15q13.3 deletions have been associated with a severe phenotype.<sup>43</sup>

We did identify CNVs at several other hotspot regions that have been associated with neurocognitive disorders. Interestingly, 2 individuals in the EE cohort have duplications of proximal 16p11.2 (chr16: 29.5–30.0Mb), which we considered to be likely pathogenic. T16335 had West syndrome and autism spectrum disorder and T2547 had multifocal epileptic encephalopathy. Duplications of this region have been associated with ID, schizophrenia, and autism.<sup>44–46</sup> We previously reported one individual with JME and the same duplication,<sup>7</sup> and Bedoyan and colleagues<sup>47</sup> reported a de novo duplication of this region in a child with EE that the authors felt was consistent with MPSI. We did not find this duplication in 11 cases with MPSI. We were able to evaluate relatives of case T16335 and found that the duplication was also present in the proband's sister who also had West syndrome, and father, both of whom had autism spectrum disorder. Similar to the deletions of 15q13.3, 15q11.2, and 16p13.11, duplications at 16p11.2 appear to be a risk factor for a wide range of neurocognitive and neuropsychiatric disorders, including different types of epilepsy, autism, ID, and schizophrenia, though the mechanism underlying such highly variable expression is not yet known.<sup>48,49</sup> As discussed in the next section, we also identified a deletion of distal 16p11.2 in a patient with a second deletion.

### Two Hits

Four patients (1.3% of cohort) in our series carry 2 rare CNVs (16% of patients with CNVs, similar to controls in which 29% of patients with rare CNVs have carry at least 2;  $p = 0.18$ ). Patient T18349 carries 2 deletions: a deletion of 16p11.2 (chr16:28.8–29.0Mb) and a deletion of the *SLC1A3* gene. The 16p11.2 deletion has been associated with variable phenotypes including early-onset obesity<sup>50</sup> and variable developmental delay,<sup>51</sup> with a mi-

nority of patients reported to have seizures. Interestingly our patient was also obese. A de novo missense mutation in *SLC1A3* has been reported in 1 patient with episodic ataxia, seizures, and hemiplegia,<sup>52</sup> and a different missense mutation segregates with a mild form of episodic ataxia in another family.<sup>53</sup> Given that both the 16p11.2 deletion and the *SLC1A3* gene have been associated with epilepsy, it is possible that the 2 hits together in this patient result in a more severe phenotype of SGE with abnormal early development and moderate ID.<sup>54</sup>

Patient T2709 with MPSI has a maternally inherited deletion encompassing the entire *LRRK2* gene and a paternally inherited deletion involving the *SCLT1* gene. *SCLT1* acts as a linker protein between the voltage-gated sodium channel  $Na_v1.8$  and clathrin.<sup>55</sup> *LRRK2* is a brain-expressed serine/threonine protein kinase in which gain-of-function mutations cause autosomal dominant early-onset Parkinson disease.<sup>56</sup> We hypothesized that complete loss of function of *LRRK2* might lead to the MPSI phenotype in our patient, but we were unable to detect a mutation in the nondeleted allele (see Table 3). Although each deletion in T2709 is inherited, the combination of both may contribute to the phenotype.

Case T3729 has a 9Mb terminal deletion of chromosome 9p and an adjacent 18.5Mb duplication. The terminal deletion in our patient is similar to 1 described by Heinzen and colleagues<sup>8</sup> in a patient with unclassified epilepsy, developmental delay, dysmorphic features, and spastic quadriplegia. Finally, Patient T3467 with CSWS and her affected brother both have 2 duplications. One involves the distal part of the common 22q11 deletion syndrome region. The second duplication involves the 3' end of the *DOK5* gene. However, both duplications are inherited from their unaffected mother, making it less likely that either is a highly penetrant pathogenic CNV.

### Excess of Large Deletions

In our cohort, we find 12 CNVs that are >1Mb in 11 (3.5%) individuals (see Table 2). Of these, 7 (2.2%) have a large deletion, 3 (1.0%) have a large duplication, and 1 patient has 1 of each. In contrast, in a set of 2,493 control individuals, Itsara and colleagues<sup>16</sup> found that 1.6% of controls had a CNV > 1Mb; 0.3% had a large deletion, and 1.3% had a large duplication. Therefore, we find a clear excess of large deletions in our cohort (8/315 patients vs 8/2493 controls,  $p = 0.00013$ , Fisher's exact test). Our findings are similar to those of Heinzen and colleagues,<sup>8</sup> who also noted an excess of large (>1Mb) deletions in their cohort of patients with primarily focal epilepsies, compared to controls without ID.

### Comparison with Previous Studies of Epilepsies

We compared our CNVs in the EE to those of 2 recently published studies of epilepsy in which whole-genome CNV analysis was performed.<sup>7,8</sup> Heinzen and colleagues<sup>8</sup> reported 36 patients with rare deletions >1Mb in a series of 3,812 patients with focal (>90%) or generalized (<10%) epilepsy. At least 3 have significant overlap with CNVs in our study: 5q34 deletion, 9p24.3–p23 deletion, and 7q21.1–q21.13 deletion. We also find some overlapping CNVs with those from our recent report<sup>7</sup> of CNVs in 517 patients with generalized (n = 399), focal (n = 63), or other (n = 55) epilepsies: 16p11.2 duplication, 5q33.2 deletion, and intragenic deletion of *CNTNAP2*. Together, these reports confirm the importance of rare CNVs in the genetic etiology of the epilepsies and emphasize the need for the evaluation of larger series of patients to better understand the pathogenic significance, especially for rare inherited CNVs.

### Conclusions

In summary, this is the first report of genome-wide CNV discovery in a large series of patients with EEs. We identified rare CNVs in 7.9% of 315 patients, at least one-half of which are likely to be pathogenic and which largely differ from CNVs found in milder epilepsies. Our results also illuminate several novel candidate genes for epilepsy in humans that deserve further study, including *CACNA2D1*, *SLC1A3*, and *ADAM23*. Comprehensive sequence analysis of these and other candidate genes in extended cohorts will help determine whether point mutations contribute to the genetic etiology of each EE.

We suggest that array CGH should be considered in the genetic evaluation of this patient population, in whom the occurrence of severe epilepsy and regression often prompts extensive and frequently negative investigations. While the interpretation of array CGH results in the clinical setting can be complicated by results of unclear significance, the application of established guidelines can facilitate interpretation and counseling.<sup>3</sup> Furthermore, as this tool is applied more widely to epilepsy cohorts in both the clinic and the research laboratory, comparison of CNVs across studies will clarify which specific CNVs are clearly pathogenic and begin to illuminate novel genes, pathways and syndromes in the epileptic encephalopathies.

### Acknowledgments

This research was supported by the NIH (NINDS 1R01NS069605 to H.C.M.); Career Award for Medical Scientists from the Burroughs Wellcome Fund

(1007607.01 to H.C.M.); and the National Health and Medical Research Council of Australia (400121 to S.F.B. and I.E.S.). E.E.E is an investigator of Howard Hughes Medical Institute.

We thank the patients and their families for participating in our research studies and referring clinicians. We thank Drs Lindsay Smith, Simon Harvey, Sophie Calvert, Kent Kelley, Frederick Andermann, and Daniel Keene for referral of patients.

### Potential conflicts of interest

E.A. has grants/grants pending and received payments for lectures from UCB Pharma Clinical Trials in Epilepsy. B.B.-Z. is employed as the head of a pediatric neurology unit and has grants/grants pending from GIF for other studies. S.B. 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 Jansen Cilag; has a patent for SCN1A testing held by Bionomics Inc and licensed to various diagnostic companies, with no financial return; was a consultant to Bionomics and Athena diagnostics over 3 years ago. J.C. and E.G. received grants from the NIH. E.E.E. received a grant from the Howard Hughes Medical Institute; is a consultant for and a member of the Scientific Advisory Board of Pacific Biosystems; is on the board of the Simons Foundation Autism Research Initiative (SFARI) Structural Variation Project, Yerkes National Primate Center, and International Cancer Genome Consortium, Ontario Institute of Cancer Research; has received various payments for lectures including service on speakers bureaus; Payment for development of the educational presentations (Henry Stewart Talks); and has stock/stock options for Pacific Biosystems. J.M. and S.Y. have received grants from the National Health and Medical Research Council of Australia. H.M. has received a grant from the NIH/NINDS (1R01NS069605); received support for travel to meetings for the study or other purposes from Sanger Center (Invited speaker, Genomic Disorders 2011 - The Genomics of Rare Diseases) and NIH/NINDS (Invited participant, Genetics of Epilepsy Workshop); and was a consultant for the Simons Foundation (SFARI Gene Advisory Board). L.S. has received grants from the Health and Research Council of New Zealand and Cure Kids (Child Health Research Foundation); and received support for travel to meetings for the study or other purposes from the International League Against Epilepsy

(ILAE classification task force). I.S. was on the Asian Oceanian Board of Janssen-Cilag; received payment for lectures including service on speakers bureaus from UCB, Janssen-Cilag, Athena Diagnostics, and Eli-Lilly; has a patent pending WO61/010176, Therapeutic Compound (filed 2008); has received payment for development of educational presentations for Athena Diagnostics; has received travel/accommodations/meeting expenses unrelated to activities listed from UCB, Biocodex, and Athena Diagnostics.

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