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### **Mutations in the GABA Transporter SLC6A1 Cause Epilepsy with Myoclonic-Atonic Seizures.**

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# Mutations in the GABA Transporter *SLC6A1* Cause Epilepsy with Myoclonic-Atonic Seizures

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GAT-1, encoded by *SLC6A1*, is one of the major gamma-aminobutyric acid (GABA) transporters in the brain and is responsible for re-uptake of GABA from the synapse. In this study, targeted resequencing of 644 individuals with epileptic encephalopathies led to the identification of six *SLC6A1* mutations in seven individuals, all of whom have epilepsy with myoclonic-atic seizures (MAE). We describe two truncations and four missense alterations, all of which most likely lead to loss of function of GAT-1 and thus reduced GABA re-uptake from the synapse. These individuals share many of the electrophysiological properties of Gat1-deficient mice, including spontaneous spike-wave discharges. Overall, pathogenic mutations occurred in 6/160 individuals with MAE, accounting for ~4% of unsolved MAE cases.

*SLC6A1* (MIM 137165) encodes GAT-1, a voltage-dependent gamma-aminobutyric acid (GABA) transporter that is responsible for the re-uptake of GABA from the synapse. GABA is the principal inhibitory neurotransmitter that counterbalances neuronal excitation in the brain and disruption of this inhibitory balance can result in seizures. To date, mutations in *SLC6A1* have not been shown to cause epilepsy in humans, although mutations in other genes that cause altered GABA signaling have been reported.

Overlapping 3p25.3 microdeletions have been reported in individuals with a wide spectrum of neurodevelopmental disorders.<sup>1</sup> Here, we describe a de novo 3p25.3 deletion in an individual with myoclonic-atic epilepsy (MAE; also called myoclonic-atic epilepsy or Doose syndrome; Table 1). This 315.6-kb deletion refines the critical interval to just two genes, *SLC6A1* and *SLC6A11* (Figure S1). In addition, two single de novo *SLC6A1* mutations in a cohort of individuals with intellectual disability and autism were reported by two independent, large exome sequencing studies.<sup>2,3</sup> These molecular genetics studies, as well as the

function of GAT-1 at the synapse, suggest that *SLC6A1* is an excellent candidate gene for epileptogenesis.

To investigate the role of *SLC6A1* in the etiology of the severe infantile and childhood epilepsies, we performed targeted resequencing in 569 individuals with a range of epileptic encephalopathies. Epileptic encephalopathies are a group of infantile- and childhood-onset epilepsies characterized by multiple seizure types and developmental delay or regression; they are associated with abundant epileptiform activity, which contributes to cognitive impairment.<sup>4</sup> All individuals or their parents or legal guardians gave informed consent to participate in the study and the institutional review boards of the University of Washington, and the University of Melbourne approved this study.

We captured all 14 coding *SLC6A1* exons and at least five base pairs of flanking intronic sequences by using molecular inversion probes (MIPs); next-generation sequencing, data analysis, and variant calling were performed as described previously.<sup>5</sup> In brief, we used MIPs and 100 ng of each proband's DNA to capture all target DNA and

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performed PCR with universal primers that contained a unique 8-bp barcode on the reverse primer. Amplified PCR products from all individuals were pooled and sequenced on an Illumina HiSeq according to a 101-bp paired-end protocol. We mapped raw reads to the genome (UCSC Genome Browser hg19) by using the Burrows-Wheeler Aligner (BWA) and performed variant calling by using the Genome Analysis Toolkit (GATK). Variants that did not adhere to the following criteria were excluded from further analysis: allele balance > 0.75, quality (QUAL) < 30, quality by depth (QD) < 5, coverage < 50×, and presence in homopolymer runs ≥ 4 bp. A threshold of 50× coverage was used for this methodology given that variants below this cut-off have a high false-positive rate; this is in contrast to other technologies, such as exome sequencing, which have a threshold of ~20×. Variants were annotated with SeattleSeq (see [Web Resources](#)), and the exome aggregation consortium (ExAC) dataset (see [Web Resources](#)) was used for assessments of variant frequency in the control population.

Overall, we sequenced 90% of *SLC6A1* to a depth of at least 50× and at an average coverage of 652× across all samples ([Figure S2](#)). We performed segregation analysis in parental DNA samples for all nonsynonymous, frameshift, and splice-site variants that were not present in the ExAC set of ~61,000 exomes (see [Web Resources](#)). We performed segregation analysis on the proband and parental DNA by using Sanger sequencing with primers designed to flank the variant of interest. Maternity and paternity were confirmed with the PowerPlex S5 system (Promega) for all de novo mutations.

We identified four likely pathogenic *SLC6A1* mutations in a cohort of 569 individuals with epileptic encephalopathies ([Table 1](#), [Table S1](#), and [Figure 1](#)). All mutations adhered to the aforementioned criteria and occurred at a highly conserved nucleotide; none were present in ExAC, and each of the positions at which these mutations occurred was covered at a sequence depth of 20× or greater in the controls. In addition, amino acid changes were predicted to be damaging by one or more of the prediction tools (Polyphen2, Grantham, and SIFT; see [Web Resources](#))<sup>6</sup> that we used ([Table 1](#)). Moreover, we considered these variants to be likely pathogenic on the basis that they occurred de novo in the affected individual in three cases, and in one individual the variant was inherited from an unaffected mother who was a somatic mosaic for this mutation. By using a single molecular MIP (smMIP) that targeted this mutation as described previously<sup>7</sup> in the unaffected mother, we detected four alleles with the mutant C allele and 43 alleles with the reference allele. This suggests that approximately 18% of the mother's white blood cells carry the mutant allele.

Strikingly, all individuals with *SLC6A1* mutations showed phenotypic homogeneity<sup>4</sup> ([Table 1](#)) in that MAE is characterized by the onset of myoclonic, myoclonic-atic, and atonic seizures between 7 months and 6 years

of age and the presence of generalized spike-wave or poly-spike-wave discharges. Development prior to seizures is usually normal.<sup>8</sup> In our cohort, 85/569 individuals had a diagnosis of MAE for which no molecular cause had been previously identified.<sup>5</sup> This statistically significant enrichment of *SLC6A1* mutations in MAE-affected probands (4/85) as compared to those with other epileptic encephalopathy phenotypes (0/484; p value 0.0005, Fisher's two-tailed test) prompted us to use the same methodology to screen this gene in an additional cohort of 75 individuals with MAE. In this validation cohort, we identified two additional *SLC6A1* mutations ([Table 1](#) and [Figure 1](#)). The c.578G>A (p.Trp193\*) mutation arose de novo, whereas the c.863C>T (p.Ala288Val) mutation was inherited from a mother who also had MAE. We performed further segregation analysis in the maternal grandparents and showed that the c.863C>T (p.Ala288Val) mutation arose de novo in the mother, who passed this mutation on to her affected daughter. Overall, in a cohort of 160 probands with MAE, we identified six *SLC6A1* point mutations, accounting for ~4% of previously unsolved MAE cases.

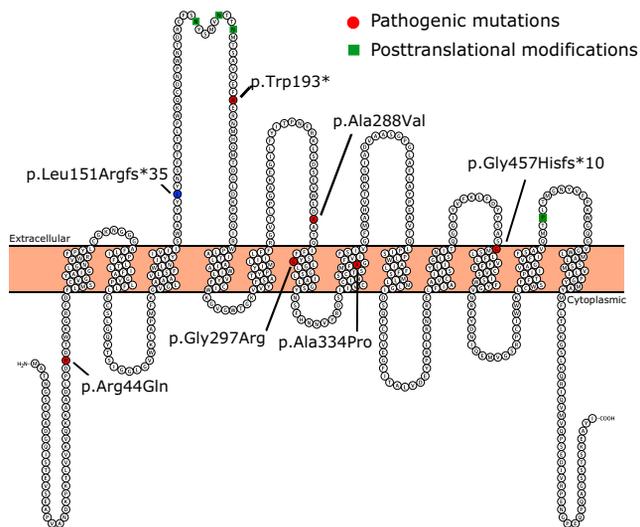
*SLC6A1* is widely expressed throughout developing and mature human, mouse, and rat brains, and its expression follows that of the GABAergic pathways.<sup>9,10</sup> GAT-1 is primarily located in the axon and nerve terminals of GABAergic interneurons, whereas GAT-3 is more abundant in astrocytes.<sup>11–13</sup> At the pre-synaptic terminal, GAT-1 is responsible for the re-uptake of GABA from the synaptic cleft. This voltage-dependent transport requires the exchange of two sodium ions and one chloride ion for each GABA molecule.<sup>14</sup> In *Gat1*-deficient mice, GABA uptake is impaired, resulting in both increased ambient GABA levels and spontaneous spike-wave discharges.<sup>15</sup>

In this study, we identified two truncating alterations (c.1369\_1370delGG [p.Gly457Hisfs\*10] and c.578G>A [p.Trp193\*]) and one partial gene deletion that most likely lead to loss of GAT-1 function. Importantly, no truncating alterations have been identified in the ~61,000 ExAC exomes, providing further evidence that loss-of-function protein changes are likely pathogenic. Mutagenesis and functional experiments at the sites of the four missense substitutions (c.131G>A [p.Arg44Gln], c.889G>A [p.Gly297Arg], c.1000G>C [p.Ala344Pro], and c.863C>T [p.Ala288Val]) suggest that they lead to a loss of GAT-1 function. Substitution at the Arg44 position has been shown to result in approximately 98% (p.Arg44Ser) and 70% (p.Arg44Lys) decreases in GABA transport activity.<sup>16</sup> We anticipate that the p.Arg44Gln substitution described here will have a similar negative effect on GABA uptake. Similarly, a p.Ala288Cys substitution reduced GABA transport activity to 5%–7% of the activity seen in wild-type-like GAT-1.<sup>17</sup> The p.Ala288Val substitution described here had the most damaging scores possible according to PolyPhen2 and SIFT and most likely results in loss of GAT-1 function. Although no mutagenesis data exist for the p.Gly297Arg alteration, Gly297, along with Ala61,

**Table 1. Clinical and Molecular Findings in Individuals with Pathogenic *SLC6A1* Mutations**

Individual	Age and Sex	Epilepsy Syndrome	cDNA Change, Protein Change, and Inheritance	GERP, CADD, PolyPhen-2, Grantham, and SIFT scores	Family History	Development prior to Seizure Onset	Age at Seizure Onset	Seizure Type at Onset
<b>Original Cohort of 569 Individuals with Epileptic Encephalopathy</b>								
1	8 years, F	MAE	c.131G>A (p.Arg44Gln), de novo	4.37, 35, 0.99 (damaging), 43, 1 (tolerated)	negative	delayed	30 months	atonic drop attacks
2	16 years, F	MAE	c.889G>A (p.Gly297Arg), de novo	4.8, 27.6, 0.37 (benign), 125, 0 (damaging)	father's first cousin has absence seizures	isolated speech delay	31 months	atonic drop attacks
3	10 years, F	MAE	c.1000G>C (p.Ala334Pro), maternally inh (9% mosaic)	5.37, 34, 1.00 (damaging), 27, 0.04 (damaging)	maternal great aunt with visual auras, paternal great uncle with GTCS, bilateral family history of speech disorders	delayed	12 months	drop attacks
4	10 years, M	MAE at 4 years, evolving to aBECTS	c.1369_1370 delGG (p.Gly457Hisfs*10), de novo	NA	negative	delayed	3 years	myoclonic-atonic, atonic seizures
<b>Validation Cohort of 75 Individuals with MAE</b>								
5	12 years, F	MAE	c.578G>A (p.Trp193*), de novo	4.86, 38, NA, NA, NA	negative	delayed	38 months	myoclonic-atonic seizures
6	22 years, F	MAE	c.863C>T (p.Ala288Val), inh from affected mother	4.98, 29.7, 1.00 (damaging), 64, 0 (damaging)	mother has MAE	delayed	14 months	myoclonic-atonic drop attacks
7 (mother)	44 years, F	MAE	c.863C>T, (p.Ala288Val), de novo	as above	negative	delayed	12 months	one febrile seizure, myoclonic-atonic seizures
<b>Novel 3p25.3 Microdeletion</b>								
3p25.3 deletion	7 years, F	MAE	deletion includes <i>SLC6A11</i> and exon 1 of <i>SLC6A1</i> , de novo	NA	negative	delayed	3 years	atonic drop attacks
<b>Previously Published Whole-Exome Sequencing Study Cases</b>								
Rauch, 2012 (ZH50743)	12 years, F	NA (cohort of individuals with ID)	c.452 delT (p.Leu151Argfs*35), de novo	NA	negative, Italian origin	delayed speech (48 months) and walking (26 months)	5.5 years	myoclonic-astatic seizures
Sanders, 2012 (13832.p1)	NA, M	NA (cohort of individuals with autism spectrum disorders)	c.863C>T (p.Ala288Val), de novo	4.55, NA, 1.00 (probably damaging), 64, 0.02 (damaging)	NA	NA	1.5 years	petit mal (absence)
Mutation coordinates based on <i>SLC6A1</i> : NM_003042.3 and protein NP_003033.3								
Genome evolutionary rate profiling (GERP) scores range from least (−12.3) to most highly (6.17) conserved residues. Combined annotation dependent depletion (CADD) Phred-scaled scores range 0–99. All PolyPhen-2 scores were calculated under the HumVar model for Mendelian disorders and ranged from 0–1, where 1 is most likely to be damaging. Grantham scores ranged from 0–215 where 215 is predicted to be most damaging. Sorting intolerant from tolerant (SIFT) scores ranged from 0–1, where 0 is predicted to be most damaging. Abbreviations are as follows: inh, inherited; F, female; M, male; aBECTS, atypical benign epilepsy with centro-temporal spikes; CSWS, continuous spike-wave discharges during slow sleep; MAE, myoclonic-atonic epilepsy; ID, intellectual disability; IPS, intermittent photic stimulation; ADD, attention deficit disorder; GTCS, generalized tonic-clonic seizures; AED, anti-epileptic drug; GSW, generalized spike wave; PSW, polyspike wave; PPR, photo-paroxysmal response; HV, hyperventilation; NA, not available; VPA, sodium valproate; LTC, lamotrigine; CLB, clobazam; CBZ, carbamazepine; LEV, levetiracetam; TPM, topiramate; ETX, ethosuxamide; KD, ketogenic diet; CZP, clonazepam. *current medication								

<b>Development after Seizure Onset</b>	<b>Other Seizure Types</b>	<b>Age at Seizure Offset</b>	<b>EEG</b>	<b>Neuroimaging</b>	<b>Other Features</b>	<b>Medications</b>
<b>Original Cohort of 569 Individuals with Epileptic Encephalopathy</b>						
plateaued, mild ID	atypical absences (onset 32 months) with blinking, myoclonic seizures (onset 2.5 years),	4 years	posterior predominant 3.5–4 Hz GSW, bilateral occipital spike-wave on eye closure, no PPR,	delayed myelination	Manual stereotypies, autistic features, hypertelorism, broad short nasal tip	CZP and VPA stopped drop attacks, VPA ceased at 5 years of age
regression at 4 years, severe ID	absences with eyelid myoclonias, myoclonic status, nonconvulsive status epilepticus	ongoing	3 Hz GSW, GPSW, PPR	normal	Autistic features, moderately severe tremor, reluctant to use hands at 14 years, aggression, thoracic scoliosis	*VPA, LTG, *CLB, LEV, TPM, *ETX; 3.5 years seizure free on CLB before seizure recurrence
moderate ID	absences with eyelid myoclonias, atonic drop attacks preceded by eyelid flutter, GTCS (onset 9 years)	ongoing; GTCS	2.5–3 Hz GSW, no PPR	normal	hyperlaxity, lumbar lordosis	VPA, LTG, *LEV, *CZP, and *ETX stopped drop attacks; KD was effective but did not completely abolish seizures
ID	absence, myoclonic seizures	6 years	2.5–3 Hz GSW, right centro-temporal region; CSWS on initial EEG, resolved	normal	autistic features, attention deficit hyperactivity	VPA and LEV, since 2012 only *LEV
<b>Validation Cohort of 75 Individuals with MAE</b>						
mild ID	absence, myoclonic seizures	3 years and 7 months	GSW, GPSW, PPR	normal	autistic features (mild)	VPA, ETX, *CLZ
regression from 2 years, moderate ID	absences, absences with eyelid myoclonias, GTCS rare	ongoing; catamenial GTCS, daily absences, myoclonic-atic seizures	.5–4 Hz GSW, PSW, atypical absences on IPS with PPR and on HV; slow background with excessive beta (drug-related)	normal	autistic features, pyramidal signs, ataxia, tremor, dyslalia, dysarthria	VPA, CBZ, LTG, CZP, CLB, LEV, TPM, ETX; benzodiazepines indispensable; CZP and CLB
regression at puberty, moderate ID	absences, absences with eyelid myoclonias, GTCS (increase with age)	ongoing; catamenial GTCS, daily absences, myoclonic-atic seizures	2.5–4.5 Hz GSW, PSW, IPS with PPR; HV provoked subclinical paroxysms	normal	oppositional behaviors (mild)	VPA, LTG, LEV, CZP, TPM
<b>Novel 3p25.3 Microdeletion</b>						
moderate ID	absences with eyelid myoclonia	ongoing	GSW, bilateral, posterior high-voltage activity	normal	hypotonia, autistic traits, absent speech	VPA
<b>Previously Published Whole-Exome Sequencing Study Cases</b>						
moderate ID (IQ < 50)	NA	NA	NA	MRI at 5 years showed mild cerebellar atrophy	autistic features, repetitive behavior, aggression, short attention span, flat and long face, large upper incisors, prognathism,	NA
delayed speech, NA then regression with loss of speech	NA	ongoing	abnormal at 2 years	MRI normal at 3 years	autism, attention deficit disorder	ADD medications, AEDs, mood stabilizers



**Figure 1. Distribution of *SLC6A1* Mutations**

A two-dimensional representation of the 12 *SLC6A1* transmembrane domains shown from 1 through 12, left to right, and predicted intracellular and extracellular domains. The six pathogenic mutations identified in this study (red), as well as the previously reported p.Leu151Argfs\*35 variant (blue) in an individual with intellectual disability and myoclonic-astatic seizures, are highlighted.<sup>3</sup> In three-dimensional space, transmembrane domains 1–5 and 6–12 are folded such that the GABA binding pocket is between transmembrane domains 1 and 6. Thus, the four pathogenic missense substitutions all cluster around the GABA binding pocket and are likely to disrupt GABA transport from the extracellular space into the pre-synaptic terminal.

Leu300, and Trp400, forms the GABA binding site.<sup>18</sup> Replacement of this small amino acid with a large positively charged residue is likely to occlude the GABA binding pocket. Finally, the p.Ala334Pro substitution occurs in transmembrane domain 7, and the presence of a large aromatic residue is likely to alter the conformation of this domain and disrupt function. In summary, although we have not performed mutagenesis studies for these specific substitutions, the evidence suggests that all six alterations could lead to a loss of function and reduced GABA uptake from the synapse, in a manner similar to that seen in *Gat1*-knockout mice.

Given that GABA is the major inhibitory neurotransmitter in the brain, it seems paradoxical that increased GABA levels would cause seizures with hypersynchronous epileptiform neuronal activity. However, an elevation in ambient and synaptic GABA, due to decreased clearance, has the capacity to enhance both phasic and tonic inhibition. Increases in either of these two modes of inhibition have been associated with the appearance of spike-wave discharges.<sup>19,20</sup> Moreover, *Gat1*-knockout mice, as well as mice administered a GAT-1 inhibitor, show spontaneous spike-wave discharges typical of absence seizures,<sup>19</sup> a seizure type seen in all individuals with *SLC6A1* mutations. Finally, tiagabine, an anti-epileptic drug that is effective in treating focal seizures and that blocks GAT-1 can cause both absence status epilepticus and myoclonic seizures in human subjects.<sup>21,22</sup>

These studies suggest that GABA function may extend beyond inhibition.

Overall, we identified six likely pathogenic *SLC6A1* mutations in seven individuals, including an affected mother and daughter, and an additional eighth individual with a deletion disrupting *SLC6A1*; all eight individuals have MAE (Table 1). The median age of seizure onset was 30.5 months (mean = 26.1 months; range = 12–38 months). All of these individuals had absence seizures, notably including eyelid myoclonia in four cases. All individuals also had drop attacks, which were myoclonic atonic in four individuals and atonic in the other four. Recording myoclonic-atonic seizures in a child with MAE can be challenging because formal video-EEG monitoring has shown that myoclonic, atonic, and myoclonic-atonic seizures can all occur in a single individual and can be hard to differentiate.<sup>8</sup> All individuals had generalized spike-waves >2.5 Hz on their EEGs, and four had a photoparoxysmal response. Seizures settled in three of the individuals, all children between the ages of 3 and 8 years; the remaining five individuals, aged 7 to 44 years, had ongoing seizures.

Although the overall electroclinical pattern was consistent with MAE, atypical features were noted. Specifically, preceding developmental delay, which can occur in a minority of individuals with MAE, occurred in all eight individuals here. Developmental slowing or regression occurred in four individuals. All individuals had intellectual disabilities that ranged from mild to severe. Six individuals had autistic features. Tremors were marked in two individuals, one of whom also had ataxia. Another individual had prominent manual stereotypies. Also, generalized tonic-clonic seizures are frequently observed in MAE,<sup>23–25</sup> but only three of our individuals had this seizure type; of these three, two were the mother and daughter, who had catamenial generalized tonic-clonic seizures. Seven of the eight affected individuals, including the affected mother, were female, but this might simply reflect the relatively small cohort rather than true biological significance.

Interestingly, in a large exome sequencing study that focused on gene discovery under a de novo mutation model, the c.863C>T [p.Ala288Val] substitution was identified in an individual with autism spectrum disorder (13832.p1).<sup>2</sup> The clinical features of individual 13832.p1, as noted in the Simons Foundation Autism Research Initiative (SFARI; Table 1), indicate that this individual had absence seizures, regression, and autism, perhaps reflecting an overlapping phenotype with MAE. Interestingly, the median seizure onset for the three individuals with the p.Ala288Val substitution was 14 months, which is much earlier than that of the other six individuals described here, for whom the median onset was 33.5 months. Further studies are needed to determine whether this difference correlates with an underlying biological mechanism.

We identified pathogenic *SLC6A1* mutations in 6/160 probands with MAE, suggesting that mutations in this

gene account for ~4% of individuals with this severe epilepsy syndrome and are more likely in individuals with pre-existing developmental delay. We also describe a de novo deletion in one individual, whose phenotype was strikingly similar to that observed in individuals with *SLC6A1* point mutations, despite the inclusion of the adjacent gene, *SLC6A11* (MIM 607592), in the deletion. A genetic etiology for MAE has been proposed since its initial description by Doose and is supported by family studies.<sup>26,27</sup> Family studies show that MAE can occur in a family with the familial epilepsy syndrome genetic epilepsy with febrile seizures plus (GEFS+), although sporadic cases of MAE are often seen.<sup>28</sup> Several large families with GEFS+, one of which includes an individual with MAE, have been described as having *SCN1A* (MIM 182389), *SCN1B* (MIM 600235), or *GABRG2* (MIM 137164) mutations.<sup>29–34</sup> Glucose transporter 1 deficiency has also been implicated in MAE, and *SLC2A1* (MIM 138140) mutations have been found in a small subset (4/84) of MAE-affected individuals with both inherited and de novo mutations.<sup>35</sup> More recently, the role of de novo mutations in MAE has expanded given that mutations in *GABRG2* (MIM 137164) have been identified in a single individual and mutations in *CHD2* (MIM 602119) have been identified in two individuals.<sup>5</sup> Each of these genes contributes slightly to the etiology of MAE, but they are also associated with a wide spectrum of epilepsy phenotypes ranging from benign to severe.

Mutations in *SLC6A1* seem to occur specifically in individuals presenting with MAE. Although this observation requires further validation, it is supported by the lack of mutations in the remaining 484 individuals who had other epileptic encephalopathies and were sequenced in this study, as well as in 264 probands with infantile spasms or Lennox-Gastaut syndrome.<sup>36</sup> Collectively, these findings suggest that *SLC6A1* mutations might cause a specific epilepsy syndrome: MAE that occurs in the context of abnormal early development. These early abnormalities might be due to the specific function of GAT-1 and GABA transport in the developing human brain.

### Supplemental Data

Supplemental Data include two figures and one table and can be found with this article online at <http://dx.doi.org/10.1016/j.ajhg.2015.02.016>.

### Consortia

The members of the EuroEPINOMICS Rare Epilepsy Syndrome Myoclonic-Astatic Epilepsy & Dravet working group are Albena Jordanova, Sarah von Spiczak, Hiltrud Muhle, Hande Caglayan, Katalin Sterbova, Dana Craiu, Dorota Hoffman, Anna-Elina Lehesjoki, Kaja Selmer, Christel Depienne, Johannes Lemke, Carla Marini, Renzo Guerrini, Bernd Neubauer, Tiina Talvik, Peter De Jonghe, Arvid Suls, and Eric Leguern.

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### Web Resources

The URLs for data presented herein are as follows:

Burrows-Wheeler Aligner, <http://bio-bwa.sourceforge.net/>

CADD, <http://cadd.gs.washington.edu/>

ExAC Browser, <http://exac.broadinstitute.org/>

PolyPhen-2, <http://www.genetics.bwh.harvard.edu/pph2/>

GATK, <http://www.broadinstitute.org/gatk/>

OMIM, <http://www.omim.org/>

SFARI, <https://base.sfari.org>

SeattleSeq Annotation 138, <http://snp.gs.washington.edu/SeattleSeqAnnotation138/>

SIFT, <http://sift.bii.a-star.edu.sg/>

SSC population dataset described in this study, <https://ordering.base.sfari.org/sfari-download-prepared-datasets.html>

UCSC Genome Browser, <http://genome.ucsc.edu>

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