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Supplemental Data

De Novo Mutations in *SLC1A2* and *CACNA1A* Are Important Causes of Epileptic Encephalopathies

Epi4K Consortium

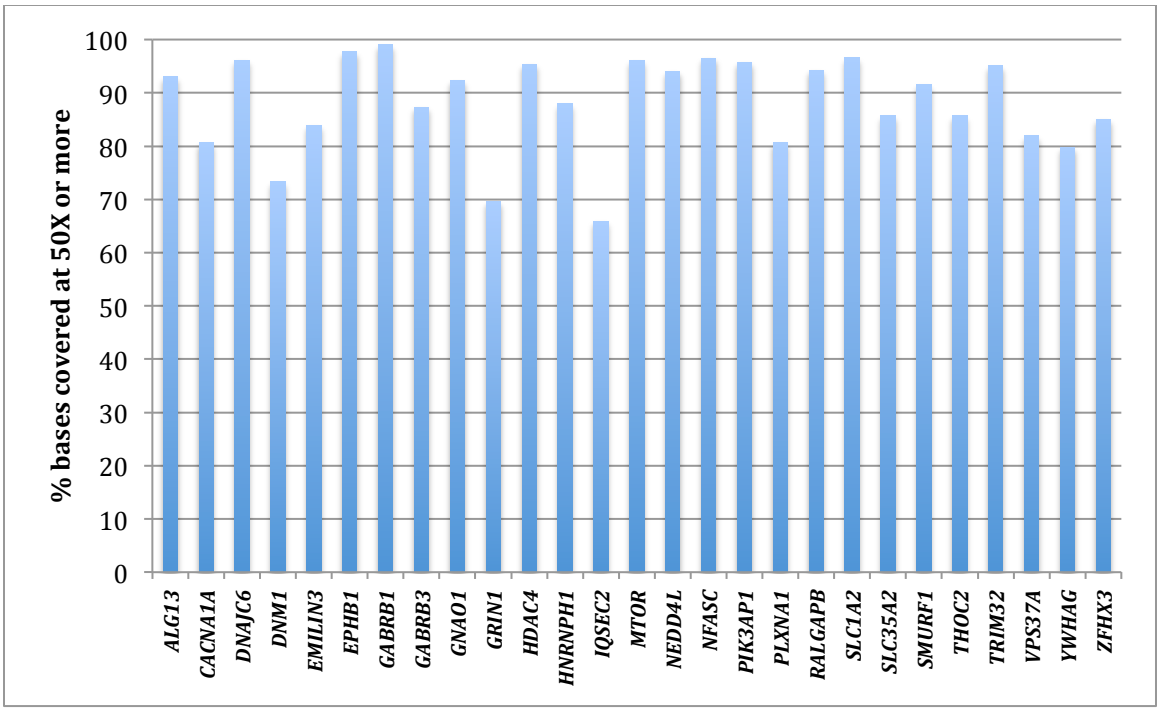


Figure S1. The average percent of the target covered at 50X or greater by gene. All coding exons plus a minimum 5 base pair intronic flank were captured and sequenced. The percent of the target covered at ≥ 50X averaged across all samples is shown. GATK was used to generate the depth of coverage statistics.

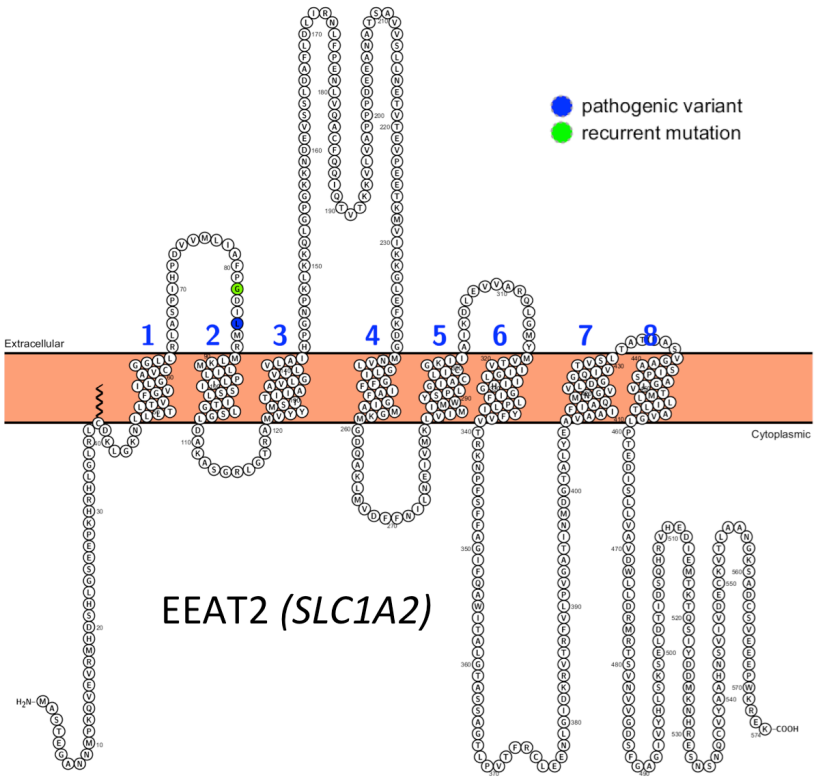


Figure S2. Schematic representation of the Excitatory Amino Acid Transporter 2 (EEAT2) encoded by *SLC1A2*. The pathogenic variant (blue; p.Leu85) and recurrent mutation (green; p.Gly82) are highlighted.

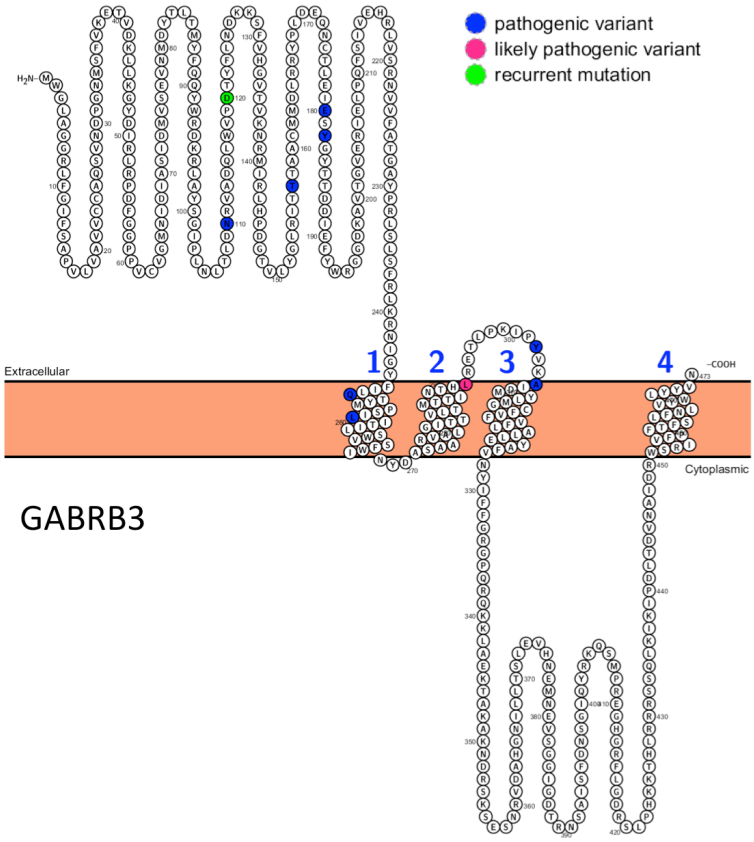
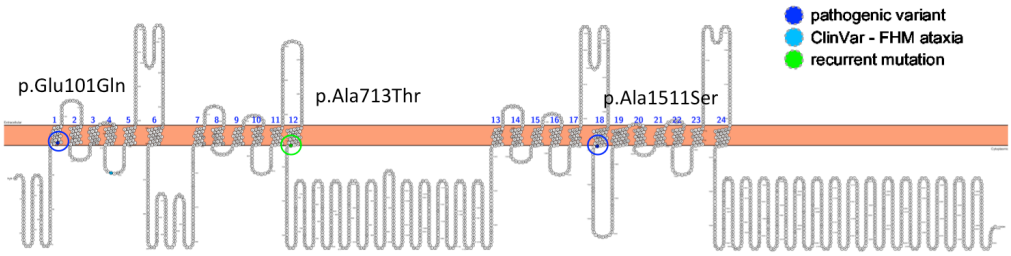


Figure S3. Schematic representation of gamma-aminobutyric acid receptor subunit beta-3 encoded by *GABRB3*. The pathogenic variants (blue; p.Asn110, p.Thr157, p. Glu180, p.Tyr182, p.Gln249, p.Leu256, p.Tyr302, p.Ala305), likely pathogenic variant (pink; p.Leu293), and recurrent mutation (green; p.Asp120) are highlighted.



Cav2.1 (*CACNA1A)*

Figure S4. Schematic representation of voltage-dependent P/Q-type calcium channel subunit alpha-1A encoded by *CACNA1A*. The pathogenic variants (blue; p.Glu101, p.Ala1511) and the recurrent mutation (green; p.Ala713) are highlighted.

Table S1. Variants of interest but unknown significance (VOUS)

Gene	Proband	Inheritance	Inferred Effect	GRCh37/hg19 Genomic Coordinate	cDNA Change	Protein Change	CADD	Diagnosis
GABRB1	T23947	Unknown	VOUS	chr4:g.47408909	NM_000812.3:c.1046A>G	p.Asn349Ser	7.46	EE
PLXNA1	T3902	Unknown, father unavailable	VOUS	chr3:g.126708005	NM_032242.3:c.569C>G	p.Pro190Arg	20.40	MAE
DNAJC6	T20772	Unknown, father not available	VOUS	chr1:g.65852559	NM_001256864.1:c.1060G>A	p.Val354Ile	12.11	FIRES
SLC1A2	T23130	Unknown, mom unavailable	VOUS	chr11:g.35302454	NM_004171.3:c.1381A>G	p.Thr461Ala	17.72	EE
ZFHX3	T22799	Unknown	VOUS	chr16:g.72821807_72821809del	NM_006885.3:c.10366_10368del	p.Phe3456del	NA	EE, malformation
CACNA1A	T25363	Unknown, father unavailable	VOUS	chr19:g.13368241	NM_023035.2:c.4525T>C	p. Phe1509Leu	19.7	EE

All variants are novel in ExAC (v0.3) and the parents where tested when available. EE, epileptic encephalopathy; MAE, epilepsy with myoclonic-atonic seizures; FIRES, febrile infection related epilepsy syndrome.

Table S2. Clinical features of individuals with mutations in *ALG13, IQSEC2, DNM1* and *GNAO1*

Identifier	Age Sex	Epilepsy syndrome	Gene	Development prior to seizure onset	Age of seizure onset	Seizure type at onset	Development after seizure onset	Other seizure types	Age of seizure offset	EEG	Neuroimaging	Other features	Medications
T22647	6y F	EOEE	ALG13	Delayed	1-2mth	Tonic	Profound ID	Spasms Myoclonic	Ongoing	Hypsarrhythmia PFA Biposterior sharp slow actvitiy	Plagiocephaly Increased signal and probable diffusion restriction in dorsal pontomedullary white matter tracts (?due to VGB)	Hypotonia Choreoathetoid movements; horizontal head movement Esotropia variable Cortical visual impairment Sleep problems	CZP, ZNS, Melatonin, Prednisolone, VGB, TPM, LEV
T17563	48y F	SGE	IQSEC2	Delayed	5y	Absence	Mild ID Regression at 5y with NCSE	Tonic-clonic Myoclonic Drop attacks	38y	Sharp slow activity maximal in temporal regions Mild background slowing	Normal	Breathholding attacks Endometriosis Mild obesity	PRM, CBZ, PB, PHT, NZP, DZP, VPA, CZP, CBZ
T24107 (sister of T26298)	4y F	EE	DNM1	Delayed	Unclear	Tonic	Profound ID	Myoclonic	Ongoing	Bilateral occipital epileptiform activity Diffuse background slowing Multifocal epileptiform discharges	Prominent CSF spaces and ventricles with dysmyelination	Dyskinesia, stereotypies exacerbated by fever, horizontal head shaking Sandifer syndrome (GORD) Cortical visual impairment Sleep problems Alternating strabismus Hypotonia	GBP, PB, LEV, steroids, nil
T26298 (brother of T24107)	23m M	EE	DNM1	Delayed	4mth	Epileptic spasms	Severe delay Regression	Focal clonic Tonic-clonic Myoclonic Focal impaired awareness	Ongoing	Bitemporal epileptiform activity	Normal	Movement disorder: forced blinking at 3 mths, complex motor behaviours, side to side rocking, facial grimace, non-integrated hyperkinetic movements of trunk and limbs Cortical visual impairment Generalised hypotonia Sleep disorder	CLB, prednisolone, LEV
T25023	26m M	EOEE	GNAO1	Increased foetal movements with feelings of "flipping" from 32w	26 minutes (clinical seizure at 9d)	Apnoeic seizures (recurrent apnoeas without respiratory aetiology) Focal clonic movements of tongue and jaw, colour change, abnormal eye movements and back arching 9d: clonic activity of L eye and side of mouth, drooling and grey colour	Delayed Regression	IS Focal Jerky movements with eye deviation Asymmetric tonic Tonic-clonic	Ongoing	Multifocal Modified Hypsarrhythmia Burst suppression in sleep High voltage midline central discharges during spasms Generalised decrement with low voltage fast activity	Moderate-severe progressive global atrophy with delayed myelination Thin CC	Strabismus (requiring surgery) Autonomic dysfunction (excessive sweating with seizures) Central hypotonia Choreoathetosis	VPA, KD, pyridoxal-5-phosphate, VGB, TPM, LEV, PB, prednisolone

ACTH, adrenocorticotropic hormone; ADHD, attention deficit hyperactivity disorder; AZD, acetazolamide; CBZ, carbamazepine; CC, corpus callosum; CLB, clobazam; CLZ, clorazepate; C-PAP, continuous positive airway pressure; CSF, cerebral spinal fluid; CT, computed tomography; CSE, convulsive status epilepticus; CZP, clonazepam; d, days; DZP, diazepam; EE, epileptic encephalopathy; EIEE, early infantile epileptic encephalopathy; EIMFS, epilepsy of infancy with migrating focal seizures; EME, early myoclonic encephalopathy; EOEE, early onset epileptic encephalopathy; ETX, ethosuximide; F, female; FBM, felbamate; FIAS, focal impaired awareness seizure; FS, febrile seizures; GBP, gabapentin; GORD, gastro-oesophageal reflux disease; GPFA, generalised paroxysmal fast activity; GSW, generalised spike wave; h, hours; ID, intellectual disability; IS, infantile spasms; KD, ketogenic diet; kg, kilograms; L, left; LCM, lacosamide; LEV, levetiracetam; LTG, lamotrigine; LGS, Lennox-Gastaut Syndrome; M, male; MAD, modified Atkins diet; MDZ, midazolam; mth, months; NCSE, non-convulsive status

epilepticus; NG, nasogastric; NICU, neonatal intensive care unit; NZP, nitrazepam; OCBZ, oxcarbazepine; PB, phenobarbitone; PFA, paroxysmal fast activity; PHT, phenytoin; PRM, primidone; PSW, polyspike wave; PEG, percutaneous endoscopic gastrostomy; R, right; RFM, rufinamide; sec, seconds; SE, status epilepticus; SGE, symptomatic generalised epilepsy; SPECT, single-photon emission computed tomography; STP, stiripentol; SSW, slow spike wave; TPM, topiramate; VGB, vigabatrin; VNS, vagus nerve stimulation; VPA, valproate; w, weeks; y, years; ZNS, zonisamide
*medications current at last follow-up underlined

Epi4K Consortium Members

Candace T. Myers[1], Jacinta M. McMahon[2], Amy L. Schneider[2], Slavé Petrovski[2,3,4], Andrew S. Allen[5], Gemma L. Carvill[1], Matthew Zemel[1], Julia E. Saykally[1], Amy J. LaCroix[1], Erin L. Heinzen[3], Georgina Hollingsworth[2], Marina Nikanorova[6], Mark Corbett[7], Jozef Gecz[8], David Coman[9], Jeremy L. Freeman[10], Sophie Calvert[11], Deepak Gill[12], Patrick Carney[13], Tally Lerman-Sagie[14], Hugo Sampaio[15], Patrick Cossette[16], Norman Delanty[17], Dennis Dlugos[18], Evan E. Eichler[19], Michael P. Epstein[20], Tracy Glauser[21], Michael R. Johnson[22], Ruben Kuzniecky[23], Anthony G. Marson[24], Terence J. O'Brien[4], Ruth Ottman[25], Steven Petrou[26], Annapurna Poduri[27], William O. Pickrell[28], Seo-Kyung Chung[28], Mark I. Rees[28], Elliott Sherr[29], Lynette G. Sadleir[30], David B. Goldstein[3], Daniel H. Lowenstein[31], Rikke S. Møller[6], Samuel F. Berkovic[2], Ingrid E. Scheffer[2,26,32,], Heather C. Mefford[1]

Affiliations

[1] Department of Pediatrics, Division of Genetic Medicine, University of Washington, Seattle, WA
[2] Epilepsy Research Centre, Department of Medicine, Austin Health, The University of Melbourne, Heidelberg, Victoria, 3084, Australia.
[3] Institute for Genomic Medicine, Columbia University, New York, New York 10032, USA
[4] Department of Medicine, Royal Melbourne Hospital, The University of Melbourne, Melbourne, Victoria, 3050, Australia
[5] Department of Biostatistics and Bioinformatics, Duke Clinical Research Institute, and Center for Human Genome Variation, Duke University Medical Center, Durham, North Carolina 27710 USA
[6] Danish Epilepsy Center, Dianalund, Denmark
[7] School of Medicine, and the Robinson Research Institute, the University of Adelaide, Adelaide, SA 5000, Australia
[8] School of Medicine, and the Robinson Research Institute, the University of Adelaide, Adelaide, SA 5000, Australia
[9] Neurosciences Department, The Lady Cilento Children's Hospital, Brisbane, School of Medicine, The University of Queensland, Brisbane, School of Medicine, Griffith University, Gold Coast
[10] Murdoch Childrens Research Institute, University of Melbourne, Australia
[11] Children's Health Queensland Hospital and Health Service
[12] TY Nelson Department of Neurology, The Children's Hospital at Westmead, Sydney, Australia
[13] The Florey Institute of Neuroscience and Mental Health, Austin Campus, Heidelberg, Victoria, Australia, Austin Health, Heidelberg, Victoria, Australia
[14] Pediatric Neurology Unit, Wolfson Medical Center, Holon, and Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel
[15] Department of Paediatric Neurology, Sydney Children's Hospital, Sydney, Australia
[16] Centre of Excellence in Neuromics and CHUM Research Center, Université de Montréal, CHUM-Hôpital Notre-Dam Montréal, Quebec H2L 4M1e, Canada
[17] Department of Neurology, Beaumont Hospital and Royal College of Surgeons, Dublin 9 Ireland
[18] Departments of Neurology and Pediatrics, The Children's Hospital of Philadelphia, Perelman School of Medicine at the University of Pennsylvania
[19] Department of Genome Sciences, University of Washington School of Medicine, Seattle, WA 98195, USA & Howard Hughes Medical Institute, Seattle, WA 98195, USA
[20] Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia 30322, USA.
[21] Division of Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio 45229 USA
[22] Centre for Clinical Translation Division of Brain Sciences, Imperial College London, London, SW7 2AZ United Kingdom.
[23] NYU Epilepsy Center, Depart of Neurology, New York University
[24] Department of Molecular and Clinical Pharmacology, University of Liverpool, Clinical Sciences Centre, Lower Lane, Liverpool, L9 7LJ, United Kingdom.
[25] Departments of Epidemiology and Neurology, and the G.H. Sergievsky Center, Columbia University; and Division of Epidemiology, New York State Psychiatric Institute, New York, New York 10032 USA.
[26] Florey Institute of Neuroscience and Mental Health, The University of Melbourne, VIC 3010, Australia.
[27] Division of Epilepsy and Clinical Neurophysiology, Department of Neurology Boston Children's Hospital, Boston, Massachusetts 02115 USA.

[28] Neurology and Molecular Neuroscience Research, Institute of Life Science, Swansea University Medical School, Swansea University SA2 8PP, United Kingdom
[29] Departments of Neurology, Pediatrics and Institute of Human Genetics, University of California, San Francisco, San Francisco, California 94158 USA.
[30] Department of Paediatrics and Child Health, University of Otago, Wellington, New Zealand
[31] Department of Neurology, University of California, San Francisco, San Francisco, California 94143 USA
[32] Department of Paediatrics, Royal Children's Hospital, The University of Melbourne, Parkville, Victoria, 3050, Australia