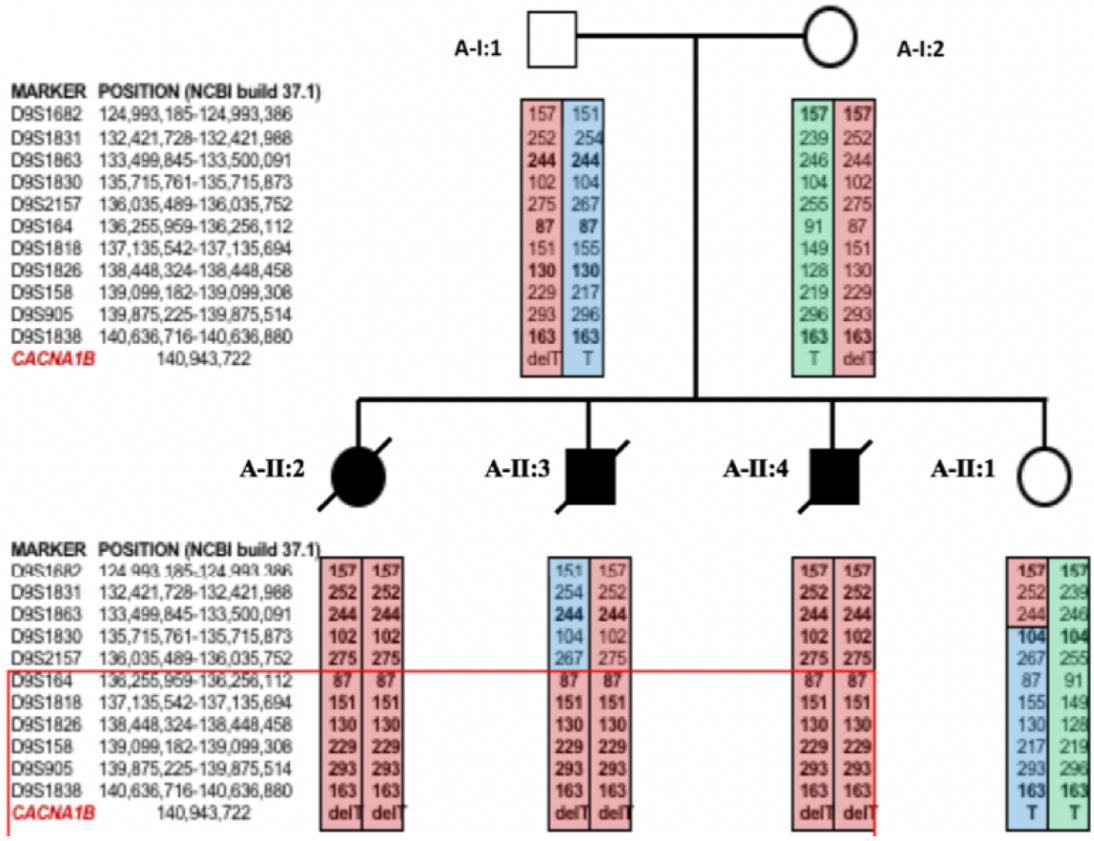
Supplemental Data.

Figure S1 : Microsatellite markers analysis and narrowing of homozygous regions in Family A

Chr	Marker	Base position	A-I:1		A-I:2		A-II:2		A-II:3		A-II:4		A-II:1	
1	D1S2726	111,184,265-111,184,542	279	279	279	279	279	279	279	279	279	279	279	279
1	D1S2809	111,414,360-111,414,496	136	136	138	136	136	136	136	136	136	136	136	138
1	D1S502	112,507,911-112,508,168	275	264	271	264	264	264	264	264	264	264	264	271
1	D1S2756	113,255,639-113,255,831	193	193	193	193	193	193	193	193	193	193	193	193
1	D1S2881	114,715,330-114,715,542	208	215	191	215	215	215	215	215	215	215	215	191
1	D1S2852	115,354,165-115,354,442	265	270	246	270	270	270	270	270	270	270	270	246
1	D1S252	117,556,772-117,556,878	103	93	89	93	93	93	93	93	93	93	93	93
1	D1S189	116,693,861-116,694,071	129	133	123	133	133	133	133	133	133	133	133	123
1	D1S2863	119,077,373-119,077,572	194	194	190	194	194	194	194	194	194	194	194	194
1	D1S534	119,678,264-119,678,466	209	195	195	195	195	195	195	195	195	195	195	195
1	D1S2696	120,500,423-120,500,589	168	168	170	168	168	168	168	168	168	168	168	168
1	D1S442	145,630,507-145,630,738	234	228	230	228	228	228	228	228	228	228	228	228
1	D1S498	151,301,564-151,301,754	195	193	189	193	193	193	193	193	193	193	193	193
2	D2S2268	215,168-215,383	217	219	219	219	219	219	219	219	219	219	217	219
2	D2S2980	935,517-935,757	226	270	240	270	270	270	270	270	270	270	226	240
2	TPO	*1,524,598-1,524,783*	111	111	111	111	111	111	111	111	111	111	111	111
2	D2S323	2,106,325-2,106,515	146	142	142	142	142	142	142	142	142	142	146	142
2	D2S2393	2,718,573-2,718,818	259	231	257	231	231	231	231	231	231	231	259	257
2	D2S319	3,427,064-3,427,191	131	131	135	131	131	131	131	131	131	131	131	135
2	D2S1780	3,745,090-3,745,711	316	324	311	324	324	324	324	324	324	324	316	311
2	D2S205	4,729,264-4,729,411	154	154	156	154	154	154	154	154	154	154	154	156
2	D2S2166	4,881,754-4,881,995	241	243	243	243	243	243	243	243	243	243	241	243
5	D5S410	152,775,054-152,775,292	335	335	333	335	335	335	335	335	335	335	335	335
5	D5S2026	153,867,609-153,867,721	109	109	109	109	109	109	109	109	109	109	109	109
5	D5S2112	156,682,856-156,683,161	304	304	301	304	304	304	304	304	304	304	304	304
5	D5S403	159,885,684-159,885,839	147	147	150	147	147	147	147	147	147	147	147	147
5	D5S529	160,214,060-160,214,263	200	200	200	200	200	200	200	200	200	200	200	200
5	D5S1476	160,418,267-160,418,399	129	129	126	129	129	129	129	129	129	129	129	129
5	D5S1386	160,649,430-160,649,788	363	363	363	363	363	363	363	363	363	363	363	363
5	D5S1955	160,738,969-160,739,159	190	190	190	190	190	190	190	190	190	190	190	190
5	D5S2118	160,754,006-160,754,258	252	252	252	252	252	252	252	252	252	252	252	252
5	C51612	*161,296,587-161,297,040*	308	308	298	308	308	308	308	308	308	308	308	308
5	C51615	*161,538,494-161,538,937*	124	124	124	124	124	124	124	124	124	124	124	124
5	D5S422	162,153,895-162,154,008	110	110	127	110	110	110	110	110	110	110	110	110
9	D9S1682	124,993,185-124,993,386	151	157	157	157	157	157	151	157	157	157	157	157
9	D9S1831	132,421,728-132,421,988	254	252	239	252	252	252	254	252	252	252	252	239
9	D9S1863	133,499,845-133,500,091	244	244	246	244	244	244	244	244	244	244	244	246
9	D9S1830	135,715,761-135,715,873	104	102	104	102	102	102	104	102	102	102	104	104
9	D9S2157	136,035,489-136,035,752	267	275	255	275	275	275	267	275	275	275	267	255
9	D9S164	136,255,959-136,256,112	87	87	91	87	87	87	87	87	87	87	87	91
9	D9S1818	137,135,542-137,135,694	155	151	149	151	151	151	151	151	151	151	155	149
9	D9S1826	138,448,324-138,448,458	130	130	128	130	130	130	130	130	130	130	130	128
9	D9S158	139,099,182-139,099,308	217	229	219	229	229	229	229	229	229	229	217	219
9	D9S905	139,875,225-139,875,514	296	293	296	293	293	293	293	293	293	293	296	293
9	D9S1838	140,636,716-140,636,880	163	163	163	163	163	163	163	163	163	163	163	163
14	D14S293F	103,454,025-103,454,179	154	152	152	152	152	152	154	152	152	152	154	152
14	D14S543	104,588,845-104,589,099	257	257	242	257	257	257	257	242	257	257	257	257
14	D14S1007	105,977,978-105,978,100	118	110	120	110	110	110	118	120	110	110	118	110
20	D20S905	5,863,629-5,863,717	85	90	85	90	85	90	85	90	85	90	90	85
20	D20S892	6,750,125-6,750,333	204	216	216	208	204	208	204	208	204	208	216	216
20	D20S846	6,764,978-6,765,246	276	274	270	270	276	270	276	270	274	270	274	270
20	D20S448	7,162,524-7,162,772	235	224	227	224	224	224	224	224	224	224	235	227
20	D20S115	7,659,962-7,660,198	242	242	244	242	242	242	242	242	242	242	242	244
20	D20S879	8,564,923-8,565,173	252	258	252	258	258	258	258	258	258	258	258	252
20	D20S851	8,861,944-8,862,075	127	135	135	135	135	135	135	135	135	135	135	135
20	D20S175	9,195,136-9,195,303	167	169	165	169	169	169	169	169	169	169	169	165
20	D20S160	10,498,912-10,499,274			344	336	328	336			328	336	328	344
21	D21S1916	28,981,080-28,981,316	242	244	246	244	244	244	242	244	244	244	244	244
21	D21S1265	29,729,157-29,729,268	102	112	102	112	112	112	102	112	112	112	112	112
21	D21S1901	30,846,638-30,846,852	220	217	220	217	217	217	220	217	217	217	217	217
21	D21S1239	31,962,086-31,962,342	259	267	259	267	267	267	259	267	267	267	267	267
21	D21S263	32,221,933-32,222,107	221	197	204	197	197	197	221	197	197	197	197	197
21	D21S1909	32,533,316-32,533,555	243	241	243	241	241	241	243	241	241	241	241	241
21	D21S1413	33,848,103-33,848,274	153	182	153	182	182	182	153	182	182	182	182	182
22	D22S539	22,257,872-22,257,996	202	206	210	206	206	206	206	210	206	206	206	206
22	D22S686	23,068,519-23,068,722	201	205	209	205	205	205	205	205	205	205	205	205

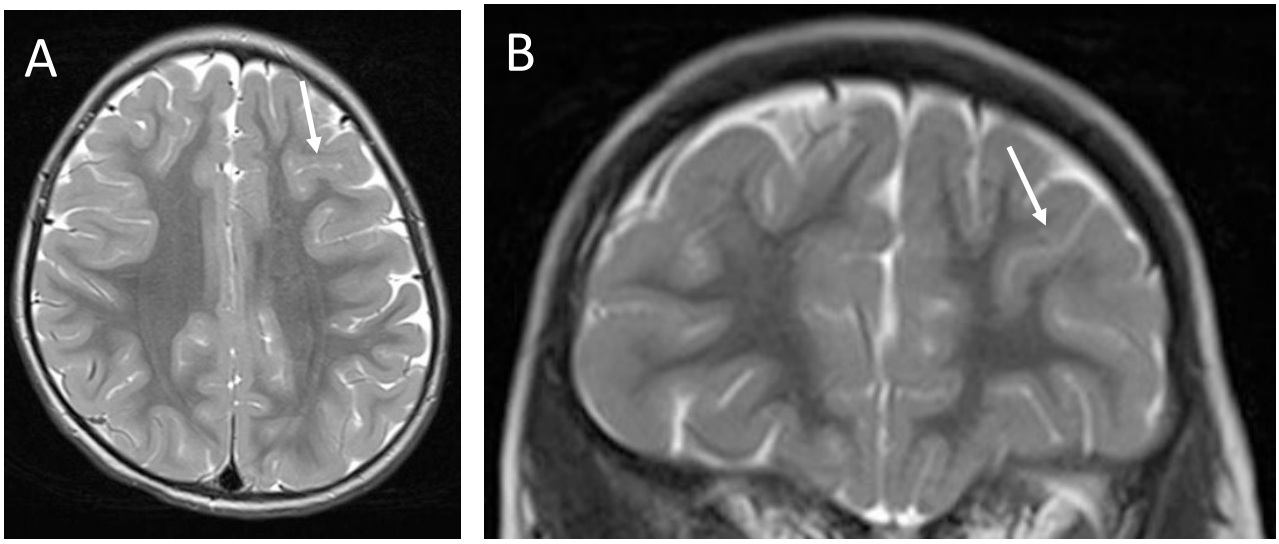
Familial genotyping using microsatellite markers narrows the common disease-associated homozygous region on chromosome 1,2 and 9 in three affected individuals (A-II:2, A-II:3 and A-II:4) by the black framed box. (NCBI Build 19)

Figure S2: Microsatellite markers analysis of homozygous region on chromosome 9



Haplotype analysis of homozygous region on chromosome 9 in Family A. Genotyping of microsatellite markers on chromosome 9q33.2-q34.3 (NCBI build 19) shows a common homozygous haplotype indicated by the red framed boxes in three affected individuals (A-II:2, A-II:3 and A-II:4).

Figure S3: MRI Brain imaging of Patient C-ii:1



T2 axial (a) and coronal (b) showing subtle asymmetry of the frontal lobes with a unilateral deep and linear appearing sulcus of the anterior left frontal lobe of unclear significance

Figure S4: *CACNA1B* brain expression profile

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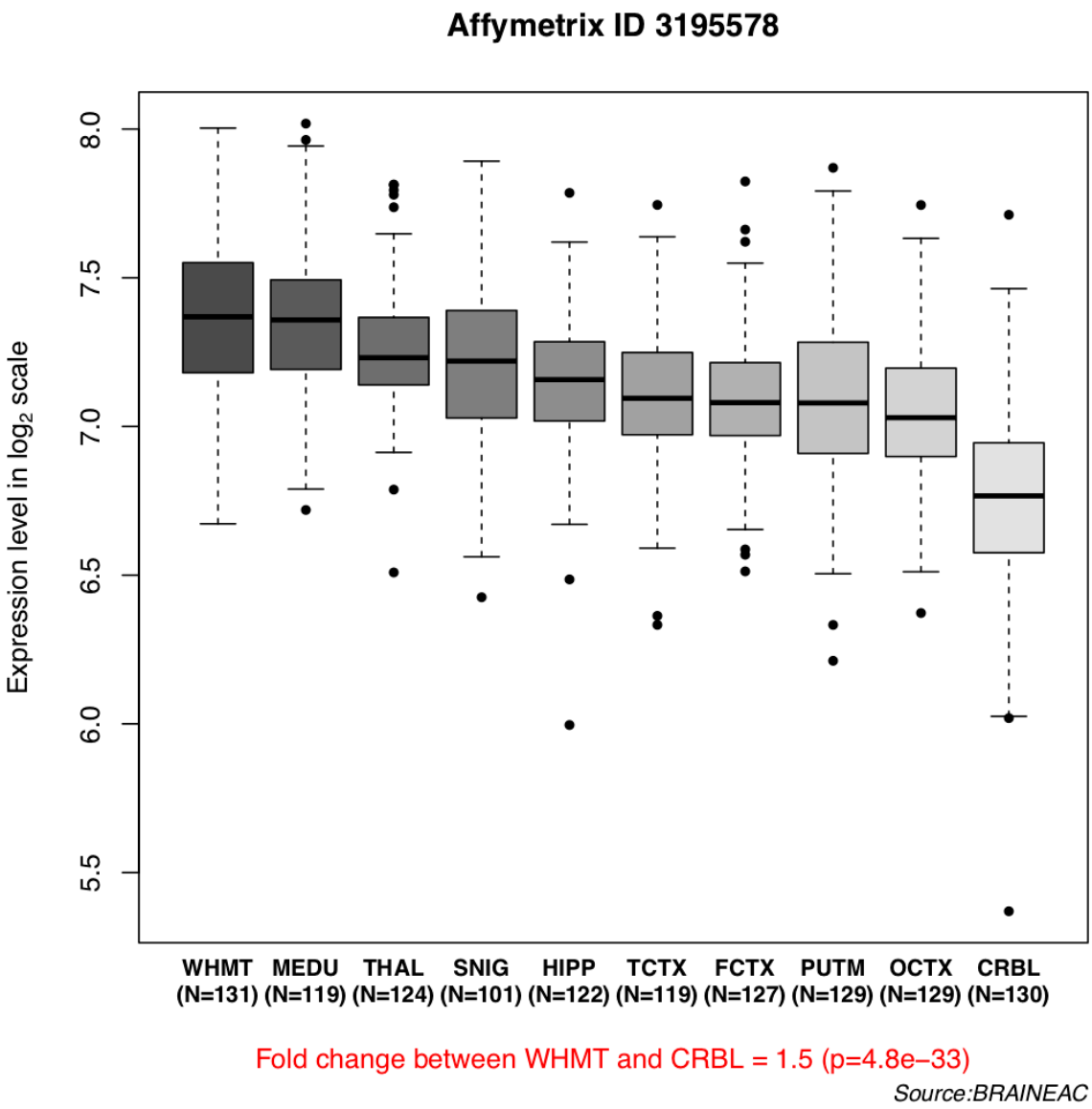
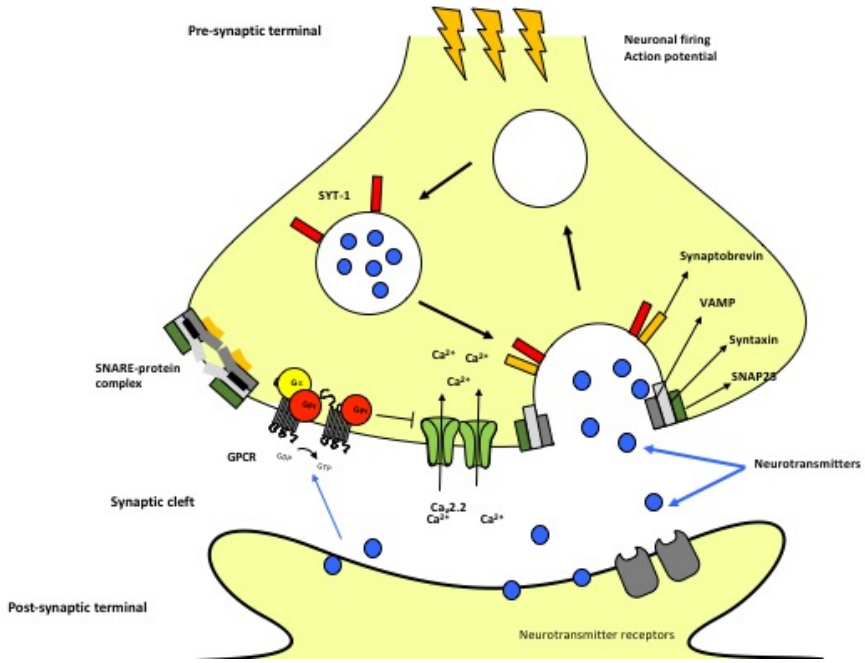


Box plots of CACNA1B mRNA expression levels in ten adult brain regions. Expression levels based on exon array experiments, plotted on a log2 scale (y axis). This dataset was generated using Affymetrix Exon 1.0 ST Arrays of brain tissue from 134 control individuals, collected by the Medical Research Council (MRC) Sudden Death Brain and Tissue Bank, Edinburgh, UK, and the Sun Health Research Institute (SHRI), an affiliate of Sun Health Corporation, USA. *CACNA1B* is ubiquitously expressed across all 10 brain regions analyzed.
Putamen (PUTM), frontal cortex (FCTX), temporal cortex (TCTX), occipital cortex (OCTX). hippocampus (HIPP), substantia nigra (SNIG), medulla (specifically inferior olivary nucleus, MEDU), intralobular white matter (WHMT), thalamus (THAL), and cerebellar cortex (CRBL). "N" indicates the number of brain samples analyzed to generate the results for each brain region. Ranges extend from the box to 1.53 the interquartile range. Source: BRAINEAC; http://www.braineac.org.

Figure S5: Voltage gated Ca+ channels and regulation of pre-synaptic neurotransmitter release



Action potentials mediate Ca^{2+} influx through $Ca_v2.2$ channels. The $Ca_v2.2$-mediated elevation of pre-synaptic Ca^{2+} triggers fusion of the secretory vesicle membrane with the plasma membrane through the primed SNARE protein complex (syntaxin [SYT-1], SNAP-25, VAMP and synaptobrevin). Neurotransmitters (blue arrow) diffuses across the synaptic cleft and binds to receptors on the postsynaptic membrane. The process is regulated by the G-Protein-coupled receptor (GPCR), Gβγ which modulates $Ca_v2.2$ and Ca^{2+} influx.

Table S1: Genetic diagnoses identified in DEE affected individuals with an epilepsy-dyskinesia phenotype

Total number of affected individuals with DEE		**494**
Total number of DEE patients with epilepsy-dyskinesia		**61 (12.3%)**
Diagnosis	**Number of Cases**	**20/61**
ATP1A3	2	
CDKL5	1	
DNM1	1	
DNM1L	1	
FOXG1	2	
MEF2C	1	
PRRT2	1	
SCN2A	4	
SCN8A	2	
SLC9A6	2	
SNORD118	1	
STXBP1	1	
Chromosomal Translocation	1	
Undiagnosed epilepsy-dyskinesia affected individuals		**41/61**

Table S2: Metabolic, genetic, radiology and elctrophysiology testing in families with biallelic mutations in *CACNA1B*

	Family A			Family B		Family C
	II:2	**II:3**	**II:4**	**II:1**	**II:2**	**II:1**
Metabolic	*Blood:* AA, ACP, BIO, carnitine, caeruloplasmin, copper, lactate, urate, VLCFA *Urine:* AA, OA, thiosulphate, *CSF:* Lactate, glycine	*Blood:* Ammonia, cholesterol, isoelectric focusing of transferrin, lactate, leucocyte gangliosidosis profile, triglycerides, uric acid *Urine:* OA, AA, oligosaccharides, MPS screen, *Skin:* Normal *Bone marrow aspirate*: Normal	*Blood:* Ammonia, BIO, lactate *CSF:* Lactate, glucose, protein, AA	*Blood:* CK, caeruloplasmin, copper isoelectric focusing of transferrin, lactate, thyroid function, VLCFA, white cell enzymes *Urine*: OA, AA, MPS, Purine, Pyrimidine *CSF*: Lactate, NT, Protein, Glucose, *Skin:* Histology normal, EM, no lafora bodies *Fibroblast:* Normal EM and PDH activity *Muscle:* Histology: numerous small atrophic fibres, respiratory chain and immunohistochemistry normal	*Blood:* BIO, CK, lactate, isoelectric focusing of transferrin, purine, pyrimidines white cell enzymes, VLCFA	*Blood:* AA, ACP, Ammonia, CK, homocysteine, lactate, B12, thyroid function *Urine:* OA
Genetics (all normal)	Karyotype	*Single gene testing: CDKL5, MELAS, MERFF, NARP (blood)*	*Single gene testing: ARX, CDKL5, ST3GAL5* 29 gene DEE panel	Karyotype and 15q methylation Microarray, - maternally inherited duplication 18p11.32 not thought to be relevant *Single gene: ARX, ATRX, FMR, MECP2, MELAS, MERFF, NARP, PLP1,PTT, TPP1*	-	Microarray 117 gene DEE panel[a]
MRI (Age)	Generalised atrophy, increase in extra-axial spaces (1y)	Asymmetry of temporal horn. Mild atrophy of right temporal lobe and prominent left sylvian fissure. Mild degree of periventricular high FLAIR (2y)	Normal (1.1y)	Normal (3.75y)	No neuroimaging	Subtle asymmetry of frontal lobes with a unilateral deep and linear appearing sulcus of the anterior left frontal lobe (4y)*
EEG	Hypssarhythmia -->LGS	MF EE (14m)→ MF EE (3.5y) MF EE bilateral epileptiform discharges (7y)	Hypssarhythmia (16m) →LGS (4y)→LGS /EE(6Y)	Diffuse slow background, continuous HA spike/sharp and slow wave (2y) MF epileptiform activity. BS during sleep (9y)	Dysrhythmic background and symmetrical sharpened activity (2.5y)	EE (4y) → EE (5y)
VEP/ERG	Normal	VEP delayed and reduced amplitude- CVI	Not undertaken	Normal	Normal ERG	Not undertaken

[a]List of DEE genes available in Table S10 * See Figure S3

Legend: Results of investigations normal unless otherwise indicated. AA, amino acids; ACP, acylcarnitine; arrayCGH, array comparative genomic hybridization; BIO, biotinidase; BS, burst-suppression; CK, creatine kinase; CSF, cerebrospinal fluid; DEE, developmental and epileptic encephalopathy; EE, epileptic encephalopathy; EM, electron microscopy; ERG, Electroretinogram; FLAIR, Fluid-attenuated inversion recovery; GER, Gastro-oesophageal reflux; HA, high-amplitude; LGS, Lennox-Gastaut syndrome; m, months; MF, multi-focal; MPS, mucopolysaccharides; NT, neurotransmitters; OA, organic acids; PDH, pyruvate dehydrogenase deficiency; UOA, urine organic acids; VLCFA, very long chain fatty acids; Y, years

Table S3: Homozygous regions identified on SNP array in Family A

Chr*	Start*	End*	first SNP	last SNP	Size (Mb)
1	111,106,576	151,809,066	rs6537672	rs11204897	40.7
2	12,994	5,292,652	rs11127467	rs16863421	5.3
5	150,578,574	162,235,216	rs3734038	rs562293	11.7
9	122,583,320	141,087,366	rs10818388	rs1820789	18.50
14	101,937,865	105,163,200	rs4906122	rs557668842	4.16
20	6,967,730	9,816,249	rs3885922	rs723118	2.85
21	27,688,357	34,408,177	rs4817105	rs762237	6.72
22	19,103,598	26,473,392	rs807743	rs6004929	7.37

*NCBI Build 37.1 Chr, chromosome
Regions excluded by microsatellite marker analysis are highlighted in grey.

Table S4: Homozygous regions identified by microsatellite marker analysis in Family A

Chr*	Start*	End*	first SNP	last SNP	Size (Mb)
1	111,184,296	117,556,772	rs757470844	rs113497537	6.37
2	215,163	4,881,995	rs761889979	rs112169262	4.67
5	150,578,574	162,235,216	rs3734038	rs562293	11.7
9	136,035,748	140,636,880	rs191830010	rs113128506	4.6
20	6,765,248	10,498,908	rs528676029	rs765195951	3.73
22	19,103,598	26,473,392	rs807743	rs6004929	7.37

*NCBI Build 37.1 Chr, chromosome

Table S5: Rare homozygous variants identified on whole exome sequencing in A-II:4 located in homozygous regions of linkage

Chr*	Gene	Change on cDNA level Protein level	NCBI Transcript ID	Mutation Type	MAF 1000G EVS GnomAD	PolyPhen-2 SIFT Provean Mutation Taster	Expression pattern	Function	Comments
1:115576654	*TSHB*	c.223A>G p.Arg75Gly	NM_000549.3	Missense	0.0024 Absent 0.001307	Benign Tolerated Deleterious Polymorphism	Pituitary gland Pancreas Connective tissues	Hormone activity	Excluded as predicted to be benign by multiple *in silico* prediction programs No brain expression Known phenotype of congenital hypothyroidism
9:140005403	*DPP7*	c.1343+5G>A	NM_013379.2	Splice	0.0010 Absent 0.0002197	- - - Polymorphism	Ubiquitous	Aminopeptidase activity Dipeptidylpeptidase activity Protein binding	Minimal effect on splicing HSF: -15.2% MaxEnt -45.8% NN splice -78.5%
9:140943722	*CANCA1B*	c.3665del p.Leu1222Argfs*29	NM_000718.2	Frameshift	Absent Absent Absent	- - - Disease causing	Pituitary Brain[a] Testis Eye Embryonic Muscle Intestine Mus	Voltage gated calcium channel activity Protein C terminus binding ATP activity Voltage gated ion activity	Candidate gene for phenotype

*NCBI Build 37.1 Chr, chromosome; MAF, mean allele frequency
[a] See Figure S4 for brain expression

Table S6: 154 genes causing developmental and epileptic encephalopathy interrogated on whole exome sequencing data

*ABAT**	*CASK*	*COL4A2**	*EML1*	*GLYCTK*	*ITPA*	*MAPK10**	*NHLRC1*	*PRODH**	*SCN9A*	*SLC9A6*	*TCF4*
ADSL	*CBL*	*CRH*	*EPG5*	*GNAO1*	*KCNA1**	*MBD5*	*NRXN1*	*PRRT2*	*SETD5*	*SMC1A*	*TPP1*
ALDH7A1	*CDKL5*	*CSNK1G1*	*EPM2A*	*GOSR2*	*KCNA2**	*MECP2*	*PCDH19*	*PURA*	*SIK1*	*SMS*	*TRAK1*
ALG11	*CHD2*	*CSTB*	*FOXG1*	*GPHN*	*KCNB1*	*MEF2C*	*PIGA*	*QARS*	*SLC12A5*	*SPTAN1**	*TSC1*
ALG13	*CHRNA2*	*CTSD*	*GABBR2**	*GRIN1*	*KCNC1*	*MFF*	*PIGQ*	*RANBP2*	*SLC13A5*	*SRPX2*	*TSC2*
ARHGEF9	*CHRNA4*	*DEPDC5*	*GABRA1*	*GRIN2A*	*KCNJ10*	*MFSD8*	*PIGT*	*RHOBTB2*	*SLC16A2*	*ST3GAL3*	*UBE2A*
ARX	*CHRNB2*	*DIAPH1*	*GABRB3*	*GRIN2B**	*KCNMA1**	*MOCS1**	*PLCB1**	*RYR*	*SLC25A1*	*STX1B**	*UBE3A*
*ATP1A2**	*CLN3*	*DNM1*	*GABRD**	*GSS*	*KCNQ2*	*MOCS2*	*PLPBP*	*SCARB2*	*SLC25A22*	*STXBP1*	*WDR45*
ATP1A3	*CLN5*	*DOCK7*	*GABRG2*	*HCN1*	*KCNQ3*	*MOGS*	*PNKP*	*SCN1A**	*SLC2A1*	*SUOX*	*WDR45B*
ATRX	*CLN6*	*DPYD*	*GAMT*	*HLCS*	*KCNT1*	*MTOR**	*PNPO*	*SCN1B*	*SLC35A2*	*SYNGAP1*	*WWOX**
BTD	*CLN8*	*DYRK1A*	*GATAD2B*	*HNRNPU**	*KCTD7*	*MT-TL1*	*POLG**	*SCN2A**	*SLC6A1*	*SYNJ1*	*ZEB2*
CACNA1A	*CNPY3*	*EEF1A2*	*GATM*	*HTRA2*	*KIF1BP*	*NACC1*	*PPT1*	*SCN2B*	*SLC6A19*	*SZT2*	
CACNA1H	*CNTNAP2**	*EFHC1**	*GLRA1*	*IDH2*	*LGI1**	*NECAP1*	*PRICKLE1*	*SCN3A*	*SLC6A5**	*TBC1D24*	
CACNB4	*COL4A1**	*EHMT1*	*GLRB**	*IQSEC2*	*MAGI2**	*NEXMIF*	*PRICKLE2**	*SCN8A**	*SLC6A8*	*TBL1XR1*	

*Reported SNP identified

Table S7: SNPs identified, mean allele frequency and ClinVar data on genes listed in Table S6

Genes	rs number	MAF GnomAD	MAF 1000 Genome	ClinVar
ABAT	rs1641010	0.5729	0.4694	Benign allele
	rs1079348	0.3001	0.35	Benign allele
ATP1A2	rs17846714	0.02967	0.278	Benign/likely benign
CNTAP2	rs3779031	0.2155	0.2214	Benign/likely benign
COL4A1	rs2275843	0.1633	0.1929	Benign
	rs598893	0.6146	0.4040	Benign
COL4A2	rs2281974	0.3761	0.4690	Benign
EFHC1	rs9349626	0.2312	0.2029	Benign/likely benign
GABBR2	rs16916507	0.2540	0.2334	Nil
GABRD	rs2229110	0.5949	0.4387	Benign
GLRB	rs41280501	0.3406	0.4912	Benign
GRIN2B	rs1806201	0.3096	0.3033	Likely benign
	rs7301328	0.4098	0.4415	Benign/likely benign
KCNA1	rs1048500	0.4902	0.4890	Likely benign
	rs1281174	0.7065	0.3758	Nil
	rs2227910	0.5286	0.4667	Likely benign
KCNA2	rs78349687	0.05251	0.0341	Benign
KCNMA1	rs1131824	0.3546	0.4060	Benign/likely benign
LGi1	rs1111820	0.9924	0.0226	Likely benign
MAGI2	rs2074641	0.8222	0.2135	Likely benign allele
	rs7812015	0.8208	0.2137	Likely benign allele
	rs1009524	0.1184	0.1268	Likely benign allele
MAPK10	rs13103861	0.1557	0.1422	Likely benign
MOCS1	rs34757428	0.06856	0.0625	Likely benign
MTOR	rs1057079	0.6736	0.4525	Nil
PLCB1	rs2076413	0.2695	0.2123	Very likely benign allele
	rs2235613	0.6084	0.3868	Benign allele
	rs2327089	0.9423	0.103	Benign allele
	rs2294597	0.2732	0.2286	Benign allele
POLG	rs10197672	0.4592	0.4351	Nil
PRICKLE2	rs2306380	0.3377	0.3023	Likely benign
PRODH	rs3216765	0.2434	0.2873	Nil
SCN1A	rs57393001	NA	NA	No impact on splicing
SCN2A	rs2121371	0.8034	0.2276	Benign
SCN8A	rs4761829	0.8451	0.2151	Benign/likely benign
	rs303815	0.6560	0.4962	Benign/likely benign
	rs60637	0.6500	0.4890	Very likely benign
SLC6A5	rs72932998	0.1597	0.0755	Benign
	rs2241941	0.3317	0.3269	Benign
	rs1443548	0.7738	0.2069	Benign
	rs1443549	0.9982	0.0064	Benign
	rs2276433	0.4573	0.4782	Benign
	rs7925597	0.9989	0.0026	Benign
SPTAN1	rs10760566	0.9905	0.0302	Very likely benign allele
	rs2227864	0.7987	0.3572	Benign allele
	rs1415568	0.9953	0.0152	Benign allele
	rs2227862	0.7893	0.3830	Benign allele
WWOX	rs8050128	0.4500	0.4722	Nil
	rs2303191	0.7475	0.2109	Benign allele

MAF, Minor allele frequency; NA, Not available

Table S8: Summary of total number of exomes interrogated for *CACNA1B* variants from collaborating centres

Collaborating Centre	Total number of exomes/genomes	Variants identified in *CACNA1B*
Deciphering Developmental Disorders (DDD) Study, UK	4,295 triomes	No biallelic variants
The UK10K Consortium and NIHR BioResource-Rare Diseases Consortium, UK	1,151 exomes/genomes	Compound heterozygous variants in B-II-1 and B-II-2 c.4857+1G>C c.3573_3574del
Specialist Pathology: Evaluating Exomes in Diagnostics (SPEED) Study, UK	659 genomes with neurodevelopmental disorder	No biallelic variants
Munich, Germany	12,000 exomes	No biallelic variants
Tubingen, Germany	2,500 exomes	No biallelic variants
EuroEPINOMICS	21 quartet genomes (sibling pairs with DEE and parents) 137 trio exomes (probands with DEE)	No biallelic variants
Dublin, Ireland	390 exomes	No biallelic variants
Rochester, USA	20 trio exomes with DEE. Included in a 187 gene panel sequenced in 36 individuals with epileptic spasms presenting before 12 months of age.	No biallelic variants
Boston USA	36 with EIMFS	No biallelic variants
Melbourne, Australia	464 exomes	No biallelic variants

Table S9: Homozygous regions identified on SNP array in Family C

Chr*	Start*	End*	first SNP	last SNP	Size (Mb)
1	15,076,483	111,475,910	rs6704226	rs12562083	96.36
1	161,963,276	187,356,089	rs148621602	rs2132421	25.392
2	75,164,805	89,129,064	rs148880616	rs1484864	13.964
2	12,047,467	46,026,097	rs13413491	rs540166896	33.979
2	95,341,388	120,045,357	rs1852300	rs13418816	24.703
2	199,268,161	234,941,290	rs6434969	rs4616477	35.673
3	62,394,557	90,485,635	rs12630374	rs115654511	28.091
3	26,532,249	39,801,185	rs73823549	rs7632048	13.2689
3	93,536,054	144,669,044	rs9756066	rs4681640	51.132
3	163,577,471	173,563,721	rs13088317	rs1028411728	9.987
4	55,430,030	62,766,970	rs6554192	rs17226314	7.336
4	174,822,905	184,364,601	rs7657936	rs4241771	9.541
5	5,380,741	31,758,951	rs16875502	rs500557	26,378
5	82,923,466	170,123,634	rs17284559	rs2221440	87.20
5	172,892,328	180,692,321	rs17075980	rs888708	7.799
9	114,764,995	141,025,040	rs12004156	rs4066697	26.260
11	10,771,524	51,563,041	rs571916387	rs371233611	40.792
11	54,794,727	127,082,673	rs1608400	rs79114322	72.287
12	10,624,526	31,278,031	rs78174381	rs4931443	20.653
12	83,197,611	115,095,711	rs7132287	rs535693997	31.867
13	105,544,216	115,095,705	rs9519509	rs71449096	9.551
14	25,271,815	64,926,621	rs9652368	rs759575776	39.654
14	95,934,126	104,756,421	rs17092460	rs56870907	8.822
15	22,752,399	61,572,216	rs58703112	rs11856888	38.819

Chr*	Start*	End*	first SNP	last SNP	Size (Mb)
15	61,632,785	68,188,894	rs7163861	rs62014309	6.556
16	30,877,542	35,220,517	rs4889630	rs113598411	4.343
17	53,481,802	77,268,153	rs4450463	rs2377392	23.786
18	57,182,907	78,014,582	rs930338456	rs12456851	20.831
20	12,242,333	26,289,925	rs2224189	rs11906869	14.047
20	29,448,858	43,311,088	rs113645559	rs4812849	13.862
21	32,780,742	43,076,296	rs2284510	rs2248865	10.296

*GRCh37/hg19

Table S10: Rare homozygous variants identified on whole exome sequencing in C-II:1

Chr*	Gene	Change on cDNA Protein level	NCBI Transcript ID	Mutation Type	MAF: 1000G EVS GnomAD	PolyPhen-2 SIFT Provean Mutation Taster CADD Score	Expression pattern	Function	Comments
1:45974544	*MMACHC*	c.506T>C p.Ile169Thr	NM_015506.2	Missense	Absent Absent Absent	Benign Tolerated Deleterious Disease causing 17.4	Endocrine Bone marrow and immune system Pancreas Intestine Testis Urological Brain	Cobalamin metabolism	Known disease of methylmalonic aciduria and homocystinuria,cb1C type (OMIM: 277400). Normal serum amino acids, homocysteine and urine organic acids. Not in homozygous region
9:140850226	*CANCA1B*	c.1147C>T p.Are383*	NM_000718.2	Frameshift	Absent Absent Absent	- - - Disease causing -	Pituitary Brain[a] Testis Eye Embryonic Muscle Intestine	Voltage gated calcium channel activity Protein C terminus binding ATP activity Voltage gated ion activity	Candidate gene for phenotype

*NCBI Build 37.1 Chr, chromosome; MAF, mean allele frequency; OMIM, online mendelian inheritance in man

[a] See Figure S4 for brain expression

Table S11: 117 genes on the developmental and epileptic encephalopathy panel performed in C:II-I

ADSL	*ALDH7A1*	*ALG13*	*ARHGEF9*	*ARX*	*ATP13A2*	*ATP1A2*	*ATP1A3*
ATP6AP2	*CACNA1A*	*CACNA2D2*	*CACNB4*	*CASK*	*CDKL5*	*CHD2*	*CHRNA2*
CHRNA4	*CHRNB2*	*CLCN4*	*CLN3*	*CNL5*	*CNL6*	*CNL8*	*CNTNAP2*
CSTB	*CSTD*	*DCX*	*DNAJC5*	*DNM1*	*DYNC1H1*	*DYRK1A*	*EEF1A2*
EFHC1	*EPM2A*	*FLNA*	*FOLR1*	*FOXG1*	*GABRA1*	*GABRB3*	*GABRG2*
GAMT	*GATM*	*GOSR2*	*GRIN1*	*GRIN2A*	*GRIN2B*	*GRN*	*HCN1*
HNRNPU	*IQSEC2*	*KANSL1*	*KCNB1*	*KCNH2*	*KCNJ10*	*KCNMA1*	*KCNQ2*
KCNQ3	*KNCT1*	*KCTD7*	*KIAA2022*	*LGI1*	*LIAS*	*MAGI2*	*MBD5*
MECP2	*MEF2C*	*MFSD8*	*NEDD4L*	*NHLRC1*	*NPR2*	*NR2F1*	*NRXN1*
PCDH19	*PIGA*	*PIGO*	*PIGV*	*PLCB1*	*PNKP*	*PNPO*	*POLG*
PPT1	*PRICKLE1*	*PRICKLE2*	*PRRT2*	*QARS*	*ROGDI*	*SCARB2*	*SCN1A*
SCN1B	*SCN2A*	*SCN5A*	*SCN8A*	*SCN9A*	*SLC13A5*	*SLC25A12*	*SLC25A22*
SLC2A1	*SLC6A1*	*SLC6A8*	*SLC9A6*	*SMC1A*	*SPTAN1*	*SPRX2*	*ST3GAL3*
ST3GAL5	*STXBP1*	*SYN1*	*TBC1D24*	*TBL1XR1*	*TCF4*	*TPP1*	*TSC1*
TSC2	*UBE3A*	*WDR45*	*WWOX*	*ZEB2*			