Supporting Online Material

Targeted resequencing in epileptic encephalopathies identifies *de novo* mutations in *CHD2* and *SYNGAP1*

Gemma L. Carvill[1], Sinéad B. Heavin[2], Simone C. Yendle[2], Jacinta M. McMahon[2], Brian J. O'Roak[3], Joseph Cook[1], Adiba Khan[1], Michael O' Dorschner [4,5], Molly Weaver[4,5], Sophie Calvert[6], Stephen Malone[6], Geoffrey Wallace[6], Thorsten Stanley[7], Ann M. E. Bye[8], Andrew Bleasel[9], Katherine B. Howell[10], Sara Kivity[11], Mark T. Mackay[10, 12, 13], Victoria Rodriguez-Casero[14], Richard Webster[15], Amos Korczyn[16], Zaid Afawi[17], Nathanel Zelnick[18], Tally Lerman-Sagie[19], Dorit Lev[19], Rikke S. Møller[20], Deepak Gill[15], Danielle M. Andrade[21], Jeremy L. Freeman[10, 12], Lynette G. Sadleir[7], Jay Shendure[3], Samuel F. Berkovic[2], Ingrid E. Scheffer[2,10, 13,22]*, Heather C. Mefford[1]*

Affiliations
1. Division of Genetic Medicine, Department of Pediatrics, University of Washington, Seattle, Washington, 98195, USA
2. Epilepsy Research Centre, Department of Medicine, University of Melbourne, Austin Health, Melbourne, Victoria, Australia
3. Department of Genome Sciences, University of Washington, Seattle, Washington, 98195, USA
4. Department of Psychiatry & Behavioral Sciences, University of Washington, Seattle, Washington, 98195, USA
5. Veteran Affairs Puget Sound Health Care System, Seattle, WA 98108, USA
6. Neurosciences Children's Health Queensland, Royal and Mater Children's Hospitals, Brisbane, Queensland, Australia
7. Department of Paediatrics, School of Medicine and Health Sciences, University of Otago, Wellington, New Zealand
8. Department of Paediatric Neurology, University of New South Wales, Sydney Children's Hospital, Sydney, New South Wales, Australia
9. Westmead Hospital, University of Sydney, Sydney, New South Wales, Australia
10. Department of Neurology, The Royal Children's Hospital, Parkville, Melbourne, Victoria, Australia
11. Epilepsy Unit, Schneider Children's Medical Center of Israel, Petach Tikvah, Israel
12. Critical Care & Neurosciences Theme, Murdoch Childrens Research Institute, Melbourne, Victoria, Australia
13. Department of Paediatrics, The University of Melbourne, The Royal Children's Hospital, Melbourne, Victoria, Australia
14. Paediatric Neurology, Monash Medical Centre, Melbourne, Victoria, Australia
15. TY Nelson Department of Neurology, The Children's Hospital at Westmead, Sydney, New South Wales, Australia
16. Department of Neurology, Tel-Aviv University, Tel-Aviv, Israel
17. Tel-Aviv University Medical School, Tel-Aviv, Israel
18. Department of Pediatrics, Carmel Medical Center, Technion Faculty of Medicine, Haifa, Israel

19. Metabolic-Neurogenetic Service, Wolfson Medical Center, Holon, and Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel
20. Danish Epilepsy Centre, Dianalund, Denmark
21. Division of Neurology, Department of Medicine, University of Toronto, Toronto Western Hospital, Krembil Neurosciences Program. Toronto, Canada
22. Florey Institute, Melbourne, Victoria, Australia

* To whom correspondence should be addressed:
Heather C. Mefford, hmefford@uw.edu;
Ingrid E. Scheffer, scheffer@unimelb.edu.au

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EE TARGETED RESEQUENCING STRATEGY
65 GENES

KNOWN
19 GENES

CANDIDATES
46 GENES

EPILEPSY-ASSOCIATED
13 GENES

CNV CANDIDATES
33 GENES

ARHGEF9 &, ARX &,
***CDKL5**&, FOXG1, **KCNQ2**,*
KCNT1, MECP2 &,
***PCDH19**^, PLCB1#,*
***PNPO**#, **SCN1A**, **SCN2A**,*
***SCN8A**, SLC25A22#,*
*SLC2A1, SPTAN1, **STXBP1**,*
UBE3A, **PNKP**#*

SYN1, ATP1A2,
*CHRNA7, GRIN2B, **MBD5**, **MEF2C**,*
***SYNGAP1,** KCNQ3, GABRD,*
***GABRG2**, SCN1B, CACNB4,*
PRICKLE1#

***CHD2**, EFCAB2, **HNRNPU**, ZNF532, ADAM22,*
ADAM23, DLGAP1, DLGAP2, DLGAP3, MAPK8,
OTX1, SEMA3A, SEMA3E, SH3GL2, EPHA6,
SLC1A1, SLC1A3, SMARCA2, CACNA2D1, APBA2,
CPLX1, SV2B, SV2A, GNAI1, SYT2, AKT3, STX1A
APBA1, CHD5, CLVS2, EPHB2, EPN1, ERBB4,

Supplementary Figure 1 Targeted resequencing strategy carried out in 500 patients with epileptic encephalopathy. Disease gene identification was conducted under a *de novo*/dominant inheritance model, except for #recessive, &X-linked recessive, ^X-linked but female restricted and *disruption of maternally expressed (paternally imprinted) genes. Genes in which we identified pathogenic variants appear in bold.

a

b

c
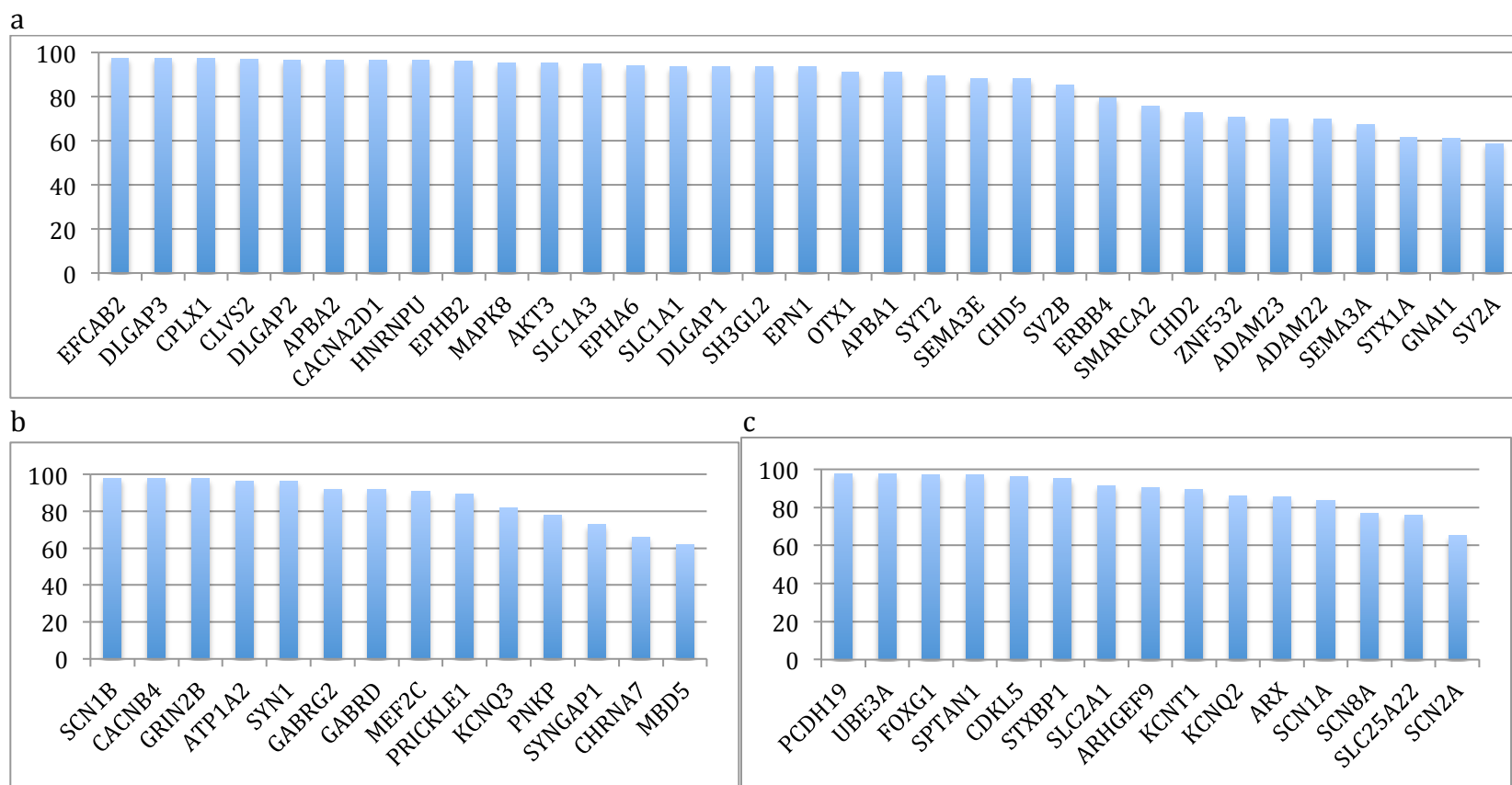
Supplementary Figure 2 Percentage of target gene resequenced at >25X for a) CNV candidate genes b) epilepsy-associated genes and c) known epileptic encephalopathy genes. GATK Depth of Coverage tool was used to calculate the percentage of exonic bases (and 5bp flanking) covered at >25X in each sample for each gene. The average coverage across all samples is reported.
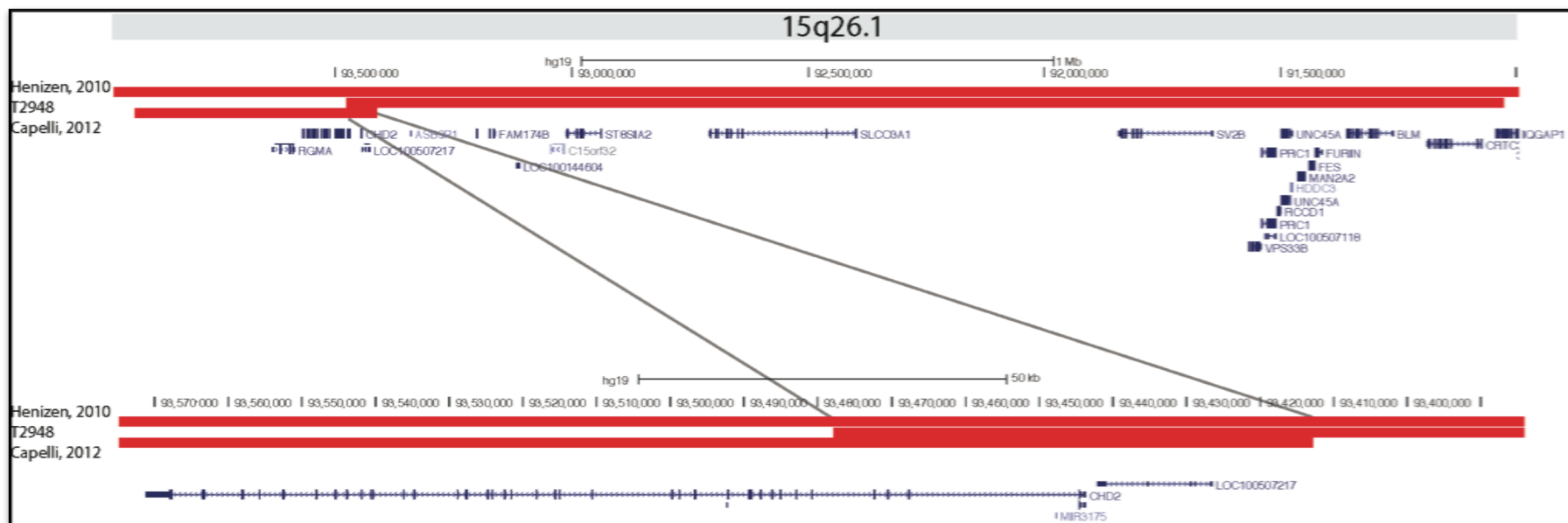
Supplementary Figure 3 Microdeletions of the 15q26.1 region encompassing *CHD2*. The *CHD2* gene and a uncharacterized non-coding RNA (LOC100507217) were the only two genes common to the two previously reported 15q26del in probands with EE [1, 2], as well as the unpublished deletion we identified in a proband (T2948) with photosensitive generalized early-onset epilepsy and ID. *CHD2* was selected as a candidate gene for mutation screening in our cohort of 500 epileptic encephalopathy probands.
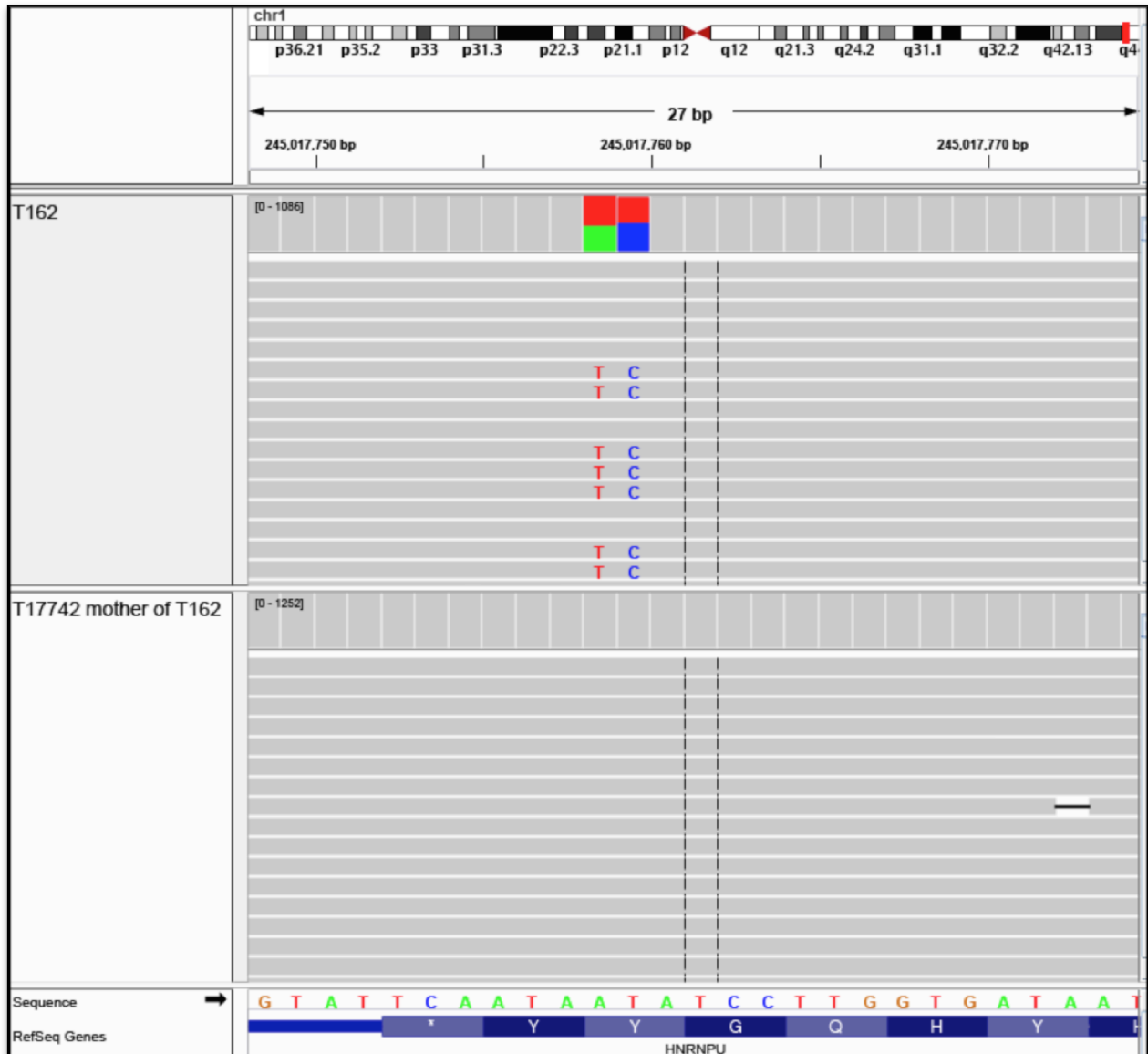
Supplementary Figure 4 The p.Y805X *HNRNPU* variant in proband T162. Output from the Integrated Genome Viewer (IGV) shows the c.471T>C and c.472A>T in the proband,T162 (top panel). Neither variant was present in the proband's mother T17742 (bottom panel).
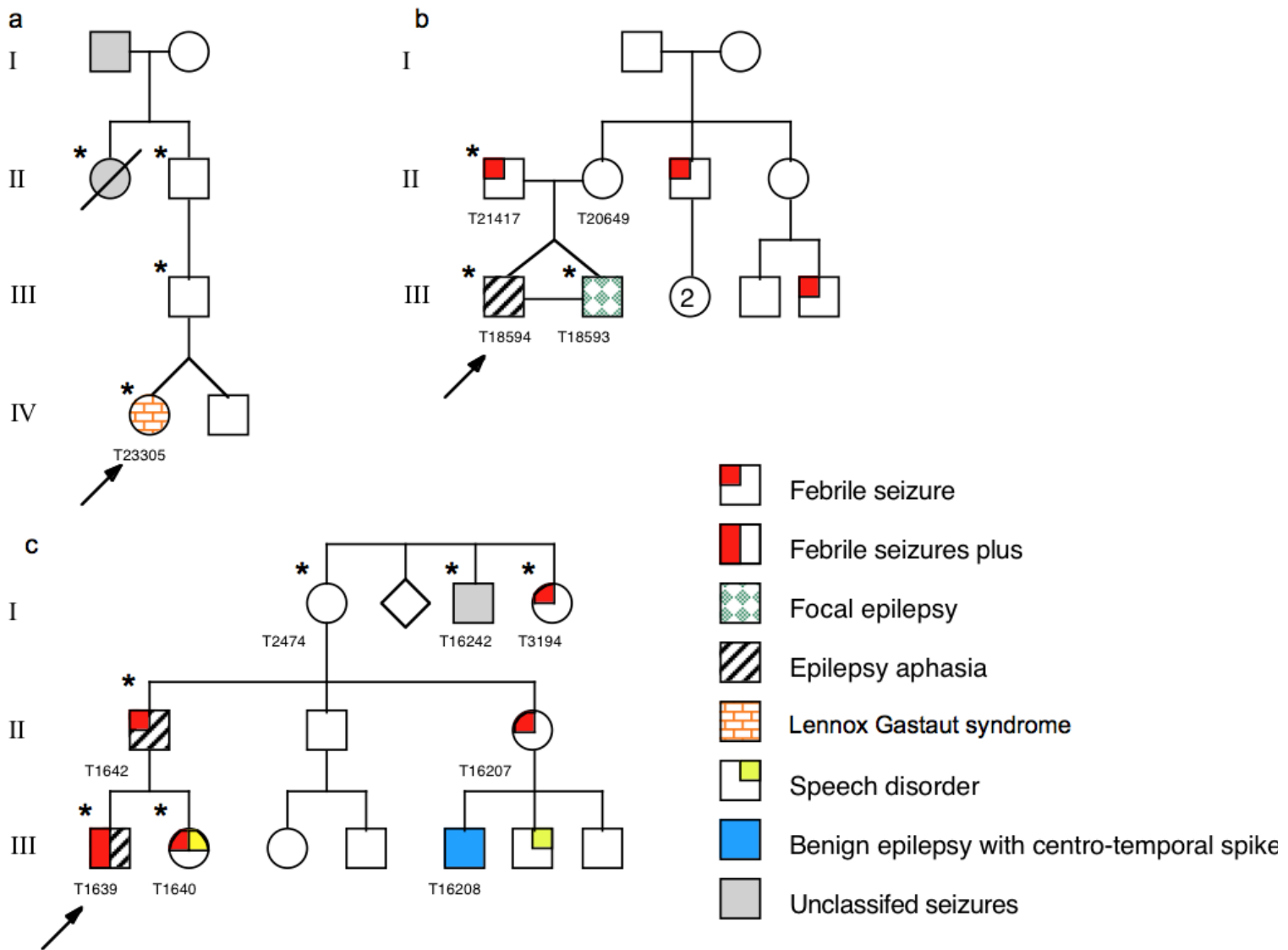
Supplementary Figure 5 Pedigrees of segregating variants in known EE genes. a) The pedigree showing the PCDH19 Arg911Gln mutation resulting in the LGS phenotype in the proband and developmental delay and seizures in her paternal great-aunt. Carrier males exhibit no phenotype, as expected for *PCDH19* mutations that cause epilepsy phenotypes in females only. b) The SCN1A Asp454Asn variant present in affected monozygotic twins as well as their father with febrile seizures. C) The Arg1988Trp SCN1A change in a proband with epilepsy aphasia and febrile seizures plus; the variant segregates with various types of epilepsy with variable expressivity and incomplete penetrance.

Supplementary Table 1 Potentially disruptive variants in known epileptic encephalopathy genes.

Proband	Gene	Position	REF/ALT	c.DNA change	Amino acid change	Inheritance
T22652	*CDKL5*	chrX:18622785	C/T	c.1741C>T	HIS581TYR	Unk*
T17329	*CDKL5*	chrX:18646566	C/T	c.2572C>T	ARG858CYS	Unk*
T24019	*KCNQ2*	chr20:62070961	G/A	c.917G>A	ALA306VAL	Unk#
T23039	*KCNQ2*	chr20:62046399	T/G	c.1298T>G	GLN461PRO	Unk*
T2542	*KCNT1*	chr9:138678206	G/A	c.3341G>A	ARG1114GLN	Unk*
T16275	*KCNT1*	chr9:138677157	C/A	c.3158C>A	PRO1053HIS	Unk*
T17135	*KCNT1*	chr9:138662266	C/T	c.1742C>T	THR581ILE	Unk*
T2509	*SCN1A*	chr2:166929953	T/C	c.179T>C	ASN60SER	Unk*
T21732	*SCN1A*	chr2:166848590	G/A	c.519G>A	PRO1732LEU	Unk*
T2529	*SCN1A*	chr2:166894315	T/C	c.291T>C	MET973VAL	Unk*
T22294	*SCN1A*	chr2:166909406	G/A	c.650G>A	THR217ILE	Unk*
T21746	*SCN1A*	chr2:166848407	G/A	c.945G>A	THR1793ILE	Unk*
T23947	*SCN1A*	chr2:166900370	G/A	c.1852G>A	ARG618CYS	Unk*
T2657	*SCN8A*	chr12:52115678	C/T	c.1984C>T	ARG662CYS	Unk#
T20187	*SCN8A*	chr12:52200885	G/A	c.5492G>A	ARG1831GLN	Unk*
T2596	*UBE3A*	chr15:25601062	G/C	c.2110G>C	PRO704ALA	Unk*

father or * neither parent available for segregation analysis

Supplementary Note: Candidate gene selection from epilepsy-associated CNVs

We considered all genes encompassed by 138 unique epilepsy-associated CNVs either from published cohorts, case reports or unpublished data [2-4]. These CNVs encompassed 1309 genes, we selected only those that were expressed in the brain (n=705). These 705 'candidate genes' were prioritized based on a number of criteria. Firstly, we compared overlapping CNVs, and defined a critical interval. For a small number of genomic regions, only one or two genes were encompassed by the overlapping intervals, and these were prioritized (n=4) (Sup Table 2) . Over larger genomic intervals that were not amenable to mapping, we performed a thorough literature search of genes in the region and applied bioinformatics tools for candidate gene prioritization, using the bioinformatics tools, ToppGene Suite [5], Endeavour [6], String [7] and the Ingenuity Pathway Analysis software (IPA) (see URLS). The ToppGene and Endeavour tools were used to rank candidate genes based on similarity to a training set of known epilepsy genes (n=115, see list below) using default settings. The ToppNett tool, was used to rank candidate genes based on relationships to a protein-protein interaction networks, also generated from the training set of genes. String was used to generate protein-protein interaction networks between the candidate and training set genes, using a high confidence threshold (0.700). Similarly, pathway analysis was conducted using IPA, with both sets of genes. Candidate genes that were consistently ranked as 'high priority' by ranking tools and/or those that were enriched within protein-protein interaction networks and canonical pathways were prioritized. Furthermore, we performed an intersection between candidate genes and those within the Mouse Genome Informatics (MGI) database that were known to generate phenotypes affecting the 'nervous system'. Finally, genes with a high burden of truncating variants in the ESP6500 (see URLs) control dataset were not selected. Using this tiered prioritization strategy, a total of 18 genes were selected for targeted resequencing. We also selected five genes that were members of the same gene family as a CNV candidate gene (homology to CNV candidate). Finally, literature searches resulted in the inclusion of six additional genes that were hypothesized as candidate genes based on function and/or animal models (literature candidate).

ToppGene Suite :http://toppgene.cchmc.org/
Endeavour: http://homes.esat.kuleuven.be/~bioiuser/endeavour/index.php
String: http://string-db.org/
MGI: http://www.informatics.jax.org/searches/allele_form.shtml

List of training set genes: *ABAT, ABCC8, ABCD1, ACOX1, ADSL, AGTR2, ALDH3A2, ALDH4A1, ALDH5A1, ALDH7A1, ALG12, ALG2, ALG6, AMT, ARG1, ARHGEF9, ARX, ATN1, ATP1A2, ATP6, ATP6AP2, ATP7A, ATRX, BRD2, C3, CACNA1A, CACNA1H, CACNB4, CASR, CDKL5, CHRNA2, CHRNA4, CHRNB2, CLCN2, CLN3, CLN8, CRH, CSTB, D2HGDH, DCX, EFHC1, ELP4, EPM2A, FLNA, FMR1, GABRA1, GABRD, GABRG2, GDI1, GFAP, GK, GPR98, GRIA3, GRIN2A, GRIN2B, HCN1, HCN2, IDS, IMPA2, KCNA1, KCNJ11, KCNMA1, KCNQ2, KCNQ3, KCTD7, KDM5C, LGI1, LGI2, MAGI2, ME2, MECP2, MED12, MEF2C, NHLRC1, OPHN1, OTC, PCDH19, PDHA1, PGK1, PHF6, PLCB1, POLG, PRICKLE1, RELN, SCARB2, SCN1A, SCN1B, SCN2A, SCN9A, SERPINI1, SEZ6, SHROOM4, SLC25A22, SLC2A1, SLC6A8, SLC9A9, SMC1A, SMS, SPTAN1, SRPX2, ST3GAL5, STRADA, STXBP1, SYN1, SYP, TBC1D24, TCF4, TRNK, TRNS1, TSC1, TSC2, TUBA1A, UBE3A, YWHAE, ZEB2*

Supplementary Table 2 Candidate gene selection details

	Gene	Genomic Location	CNV Type	Rationale	References
1.	CHD2	15q26.1	Del	Critical interval	[1-3]
2.	EFCAB2	1q44	Del	Critical interval	[3]
3.	HNRNPU	1q44	Del	Critical interval	[3, 8]
4.	ZNF532	18q21.32	Del	Only gene in interval	Unpublished
5.	ADAM22	7q21.12	Del	ToppNett, IPA, MGI	[2]
6.	ADAM23	2q33.3	Del	Endeavour, IPA, MGI	[3]
7.	DLGAP1	18p11.31	Del	ToppNett, String	[2]
8.	DLGAP2	8p23.3	none	Homology to CNV candidates	
9.	DLGAP3	1p34.3	Dup	ToppNett, String	Unpublished
10.	MAPK8	10q11.22	Del	ToppNett, String, MGI	[2]
11.	OTX1	2p15	Del	Literature candidate	[3]
12.	SEMA3A	7q21.11	Del	IPA, MGI	[2]
13.	SEMA3E	7q21.11	Del	IPA, MGI	[2, 3]
14.	SH3GL2	9p22.2	Del/Dup	Endeavour, ToppNett, String	Unpublished
15.	EPHA6	1q44	Del/Dup	String, IPA	[2, 3]
16.	SLC1A1	9q24.2	Del	ToppGene, Endeavour	[2, 3]
17.	SLC1A3	5p13.2	Del	ToppGene, Endeavour	[3]
18.	SMARCA2*	9q24.3	Del/Dup	Endeavour, ToppNett, String	[2, 3]
19.	CACNA2D1	7q21.1	Del	ToppGene, Endeavour, String	[2, 3]
20.	APBA2	15q13.1	Del	String, TopNett, MGI, Endeavour	[3]
21.	CPLX1	4p16.3	Del	ToppGene	[3]
22.	SV2B	15q26.1	Del	ToppGene, Endeavour	[2, 3]
23.	SV2A	1q21.2	none	Homology to CNV candidates	
24.	GNAI1	7q21.11	Del	ToppNett, String, MGI, IPA, Endeavour	[2, 3]
25.	SYT2	1q32.1	Dup	MGI	[3]
26.	AKT3	1q44	Del	Candidate from literature	[9]
27.	APBA1	9q21.11		Homology to CNV candidates	
28.	CHD5	1p36.31	Del	Homology to CNV candidates	
29.	CLVS2	6p22.31		Literature candidate	[10]
30.	EPHB2	1p36.12		Homology to CNV candidates	[11]
31.	EPN1	9q13.42		Literature candidate	[12]
32.	ERBB4	2q34		Literature candidate	[13]
33.	STX1A	7q11.23		Literature candidate	[14]

IPA, Ingenuity Pathway Analysis software; MGI, Mouse Genome Informatics
*SMARCA2 mutations have since been shown to cause Nicolaides Baraitser syndrome [15], SMARCA2 was selected as a CNV candidate gene prior to this publication. Similarly mutations in AKT3 have been shown to cause hemimegalencephaly [16].

Supplementary Table 3 Details of molecular inversion probes used to capture 65 target genes

MIP_name	70 mer sequence
GABRD_2	cggggtccgcgcaaacttggctcCTTCAGCTTCCCGATATCCGACGGTAGTGTcaccgcccccgaccccg
GABRD_1	cgcggggtcggggcggtggCTTCAGCTTCCCGATATCCGACGGTAGTGTagccaagtttgcgcggaccc
GABRD_3	gctacgcccgcaacttccggCTTCAGCTTCCCGATATCCGACGGTAGTGTgtggaattaaattaagtcct
GABRD_4	gctatcagcccgtccaggttggCTTCAGCTTCCCGATATCCGACGGTAGTGTgcctagaggccagcctac
GABRD_5	catggtaggtgccaccagcctctCTTCAGCTTCCCGATATCCGACGGTAGTGTggtcccatcgtggctcc
GABRD_6	gcctctgagatgtggtcgatgCTTCAGCTTCCCGATATCCGACGGTAGTGTgtgtactcctgtggaggtg
GABRD_7	gtggctgcccgacaccttcatcCTTCAGCTTCCCGATATCCGACGGTAGTGTcggcccctgtgccacctc
GABRD_8	ccagggtctcgttggtgtggttgtCTTCAGCTTCCCGATATCCGACGGTAGTGTgatcacgccgtcgggc
GABRD_9	gaaaagctcgagcggcttctgcCTTCAGCTTCCCGATATCCGACGGTAGTGTagaacaagctcatccggc
GABRD_11	gaggccagccctcccgaaagcCTTCAGCTTCCCGATATCCGACGGTAGTGTacacccactcacagctctc
GABRD_10	ggggcttctccagcagtggatgCTTCAGCTTCCCGATATCCGACGGTAGTGTcactgccatgcttgtctt
GABRD_13	gctctccgaccagtagtagacgatCTTCAGCTTCCCGATATCCGACGGTAGTGTggcacatgcggaaggg
GABRD_12	gttcaccatcaccagctaccCTTCAGCTTCCCGATATCCGACGGTAGTGTcggggcagggccgggctctg
GABRD_14	caatcctacatgccctccgtccCTTCAGCTTCCCGATATCCGACGGTAGTGTcctgagtcccatggttgg
GABRD_15	caggtggaagtgcaggctgagcCTTCAGCTTCCCGATATCCGACGGTAGTGTgaggccccgtacctagag
GABRD_16	ggggctcagcactggcctctgCTTCAGCTTCCCGATATCCGACGGTAGTGTgtcctgggtctccttctgg
GABRD_18	atcgtcagcaccgtggtgatgCTTCAGCTTCCCGATATCCGACGGTAGTGTtgaaatgagcaaaggcgta
GABRD_17	aaggcactggacgtctacttctggCTTCAGCTTCCCGATATCCGACGGTAGTGTgatggggcggcgtgag
GABRD_19	gccagggacagcactgctgaCTTCAGCTTCCCGATATCCGACGGTAGTGTatctgctatgtcttcgtgtt
GABRD_20	gccagggacagcactgctggCTTCAGCTTCCCGATATCCGACGGTAGTGTatctgctatgtcttcgtgtt
GABRD_22	cagcagagagggagaagaggacaaCTTCAGCTTCCCGATATCCGACGGTAGTGTtcctgagcgggctgcc
GABRD_21	acctgatgggctcctacaggtcgCTTCAGCTTCCCGATATCCGACGGTAGTGTcagccctgtctccccca
GABRD_23	gtttgcggccgtcaatgtcatcCTTCAGCTTCCCGATATCCGACGGTAGTGTgggtggagacaggggaga
GABRD_24	agcgcgggcgtaaatgtcaaCTTCAGCTTCCCGATATCCGACGGTAGTGTctttctcccaggaagtttct
CHD5_499	cgagacttccctgtgttgcaCTTCAGCTTCCCGATATCCGACGGTAGTGTtatgtgaccggtaggtgccc
CHD5_500	gtacatctgggaggagccgaaagcCTTCAGCTTCCCGATATCCGACGGTAGTGTgcacccgtgtgcatgc
CHD5_501	gcaacaactttgggcccaacttccCTTCAGCTTCCCGATATCCGACGGTAGTGTctcggcctgtgtgccc
CHD5_503	ggctgcagatgtcggagcgcCTTCAGCTTCCCGATATCCGACGGTAGTGTcatccctgcatcctaccatc
CHD5_502	gcagccgggtcacgtcggccttCTTCAGCTTCCCGATATCCGACGGTAGTGTggggcacacaggccgagc
CHD5_504	caggtgctggtggctctcggcCTTCAGCTTCCCGATATCCGACGGTAGTGTccctcatctacagccaaga
CHD5_505	ccaaggagtcccttgctgggaCTTCAGCTTCCCGATATCCGACGGTAGTGTgttggtcattgaggagcag
CHD5_506	caccccaggccaaggctcccCTTCAGCTTCCCGATATCCGACGGTAGTGTgtcatgttcaggtacgcggc
CHD5_507	gatcatgtaccgtgggtcattctgCTTCAGCTTCCCGATATCCGACGGTAGTGTaccgtccctccctgcc
CHD5_508	cccttcaagtctgaggtccacaaCTTCAGCTTCCCGATATCCGACGGTAGTGTgagctgcctccccctac
CHD5_510	gtgacgtatccttggccaggccCTTCAGCTTCCCGATATCCGACGGTAGTGTcctgctcaccttggcttg
CHD5_509	attttcccagaggatacagcagcCTTCAGCTTCCCGATATCCGACGGTAGTGTgctctcagagggctctc
CHD5_511	gtttcaatgggctccttctcctcCTTCAGCTTCCCGATATCCGACGGTAGTGTctctctgcccaacccc
CHD5_512	agcaaaatggtgacaaagaggaagCTTCAGCTTCCCGATATCCGACGGTAGTGTctcctgggtcccccgt
CHD5_513	cctctgcaagaaaaagagggttcCTTCAGCTTCCCGATATCCGACGGTAGTGTggaccatggggcacagg
CHD5_514	ggtgcttcctgagaaggagaagCTTCAGCTTCCCGATATCCGACGGTAGTGTtggcacctgttggactct
CHD5_515	gcttgtcctcactctccactctaCTTCAGCTTCCCGATATCCGACGGTAGTGTacatggcactggggtgg
CHD5_516	gagaggagcggccagaggagacCTTCAGCTTCCCGATATCCGACGGTAGTGTccctccgttcctggtgag
CHD5_518	gcctgtggtgtgtgtggggtgCTTCAGCTTCCCGATATCCGACGGTAGTGTgtctcagctctttcccttg
CHD5_517	atcaagggaaagagctgagacaCTTCAGCTTCCCGATATCCGACGGTAGTGTaccccacacacaccacag
CHD5_519	ggggtccgaggagatcacctcCTTCAGCTTCCCGATATCCGACGGTAGTGTagactaggggtttctgtga
CHD5_520	acctcctgccagccccgctgCTTCAGCTTCCCGATATCCGACGGTAGTGTaggttcaggagtttgagcat
CHD5_521	aagtgtcacaggcttggggtagCTTCAGCTTCCCGATATCCGACGGTAGTGTggtgctgtacttcccgtt
CHD5_522	gctcacacaggtgccgcatgaCTTCAGCTTCCCGATATCCGACGGTAGTGTccctacagagagttactca
CHD5_523	gggcggatggtgcagagaccttCTTCAGCTTCCCGATATCCGACGGTAGTGTaggagttcccctggggtc
CHD5_524	gttcagaaaggccttccgctgtCTTCAGCTTCCCGATATCCGACGGTAGTGTaggtggtcacaccctggg
CHD5_525	cccccgcaggacgccttcaacCTTCAGCTTCCCGATATCCGACGGTAGTGTcatcccagaatcctccacc
CHD5_527	cctgtctgttcccgcatctcttCTTCAGCTTCCCGATATCCGACGGTAGTGTtcccacctgaggaactgt
CHD5_526	cagttcctcaggtggggagcccagCTTCAGCTTCCCGATATCCGACGGTAGTGTtgcgggaacagacaggc
CHD5_529	cagtgttagtggtctgggctactgCTTCAGCTTCCCGATATCCGACGGTAGTGTcgtgcccctctgtctg
CHD5_528	acagaggggcacggagtgagctCTTCAGCTTCCCGATATCCGACGGTAGTGTagaccactaacactgaga
CHD5_530	gttgcgggccaggtcctcctgCTTCAGCTTCCCGATATCCGACGGTAGTGTtgacagaatcctgccccac
CHD5_531	gcaagcaggtcaactacaacgatCTTCAGCTTCCCGATATCCGACGGTAGTGTaggtggagcgggaaatc
CHD5_532	acctgacctgcccctttgcccccCTTCAGCTTCCCGATATCCGACGGTAGTGTtcccagtagtcggggtcc
CHD5_533	gtgtcatctgtagcgtcctggttcCTTCAGCTTCCCGATATCCGACGGTAGTGTggctgcggatggaggg
CHD5_534	cagaacatgaacgagtacctgagCTTCAGCTTCCCGATATCCGACGGTAGTGTcacactgcatttgcctg
CHD5_535	ccctgagacatcatgcctgtcagCTTCAGCTTCCCGATATCCGACGGTAGTGTggaggagctgaggctgg

CHD5_536	cggtcacacccatccctgatgCTTCAGCTTCCCGATATCCGACGGTAGTGTctgtatctcctggctagga
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CHD5_541	ggactggaacccgcacaatgacCTTCAGCTTCCCGATATCCGACGGTAGTGTaaacactcccatctgctg
CHD5_542	gtccagcatcttggtcatctgcCTTCAGCTTCCCGATATCCGACGGTAGTGTaatcagaacccttgggca
CHD5_543	gacttcctggagtacgaaggctaCTTCAGCTTCCCGATATCCGACGGTAGTGTgcaggcagctctctcct
CHD5_544	agacttgaccagggagcttccaCTTCAGCTTCCCGATATCCGACGGTAGTGTatgctctctgtggggtgt
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CHD5_546	cagtgcctcaaagttccgtgtgCTTCAGCTTCCCGATATCCGACGGTAGTGTagaagctgccatgatggc
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