Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Myers CT, Hollingsworth G, Muir AM, et al. Parental mosaicism in “de novo” epileptic encephalopathies. N Engl J Med 2018;378:1646-8. DOI: 10.1056/NEJMc1714579

Supplementary Appendix

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METHODS

Participants

We recruited 154 consecutive patients (and both parents) with developmental and epileptic encephalopathy (DEE) and an apparently *de novo* pathogenic variant in a known gene for DEE identified by research or clinical testing [1-3] (unpublished data) between 2003-2016, with the majority of cases identified between July 2012-August 2016. Of the 154 consecutively ascertained trios, 123 (79.9%) passed a minimum of 200 discrete captures (i.e. molecules; see below) and were included in the final analysis. Each family had at least one individual with a carefully phenotyped DEE due to an apparent *de novo* pathogenic variant in one of 33 DEE genes. Across the 33 genes, there were 121 pathogenic variants: 70 missense, 17 nonsense, 9 predicted to alter splicing, 6 small insertions and 19 small deletions (Table S4 in the Supplementary Appendix).

Genomic DNA was isolated from blood and/or saliva using standard methods; Oragene (DNA Genotek) kits were used for saliva DNA extraction. Affected siblings and parents underwent phenotyping of their epilepsy, developmental and medical history, including a validated seizure questionnaire [4]. All individuals, or parents or legal guardians of minors or those with intellectual disability, gave informed consent for study participation. The study was approved by the Human Research Ethics Committees of Austin Health, New Zealand Health and the Institutional Review Board at the University of Washington.

Mutation detection using targeted sequencing

Single molecule molecular inversion probes (smMIPs) were used to capture the genomic regions of interest as previously described [5-7] with minor modifications. The molecular tag designed within each probe identifies distinct molecules from the same individual, enabling the accurate detection and quantification of low-frequency genomic variation (i.e. mosaicism). We modified this method by using a 5-nucleotide tag, allowing us to detect mosaicism down to at least a 1% level with a minimal false discovery rate.

Library preparation followed published protocols [5] except the ratio of probe to genomic DNA was adjusted to 20,000:1. smMIPs were pooled and sequencing was performed on an Illumina HiSeq 2500 to generate 100 base pair paired-end reads. Prior to alignment, the five-nucleotide molecular tag was clipped; forward and reverse reads were merged using PEAR [8]. Merged reads were aligned to the GRCh37/hg19 reference genome using Burrow-Wheeler Alignment and analyzed using a custom pipeline available on GitHub (https://github.com/shendurelab/MIPGEN). Samtools (v1.12) mpileup was used to calculate the number of molecules assayed. Minimum mapping quality 3 (-q 3) was required for an alignment to be considered and a minimum base quality score was not a requirement for inclusion (-Q 0).

A minimum of 200 discrete captures (i.e. molecules assayed) was required for a parent to be included in the study. A parent was considered mosaic if the percentage of single molecule consensus reads exceeded 1%; each mosaic variant was confirmed in an independent capture. The variant-specific sequencing error rate was calculated for each pathogenic variant using sequencing data from all individuals unrelated to the proband (mean error rate: 0.04%). At the minimal coverage for inclusion, the assay had an 86.6% power to detect a 1% mosaic parent (*binomial test*). The median variant coverage was 826 discrete captures, allowing a 99.7% power to detect a 1% mosaic with a false discovery rate of 0.0018% (*binomial test, Bonferroni correction).*

Confirming familial relationships

We used 51 smMIPs, designed as a single nucleotide polymorphism (SNP) marker set for human identification, to test for correct relationships within our sequencing runs [9]. In cases where DNA was limited or there was low coverage in the genomic regions captured by the SNP smMIPs, microsatellite analysis using the PowerPlex S5 System (Promega) was used to confirm maternity and paternity. Parentity was confirmed for all cases.

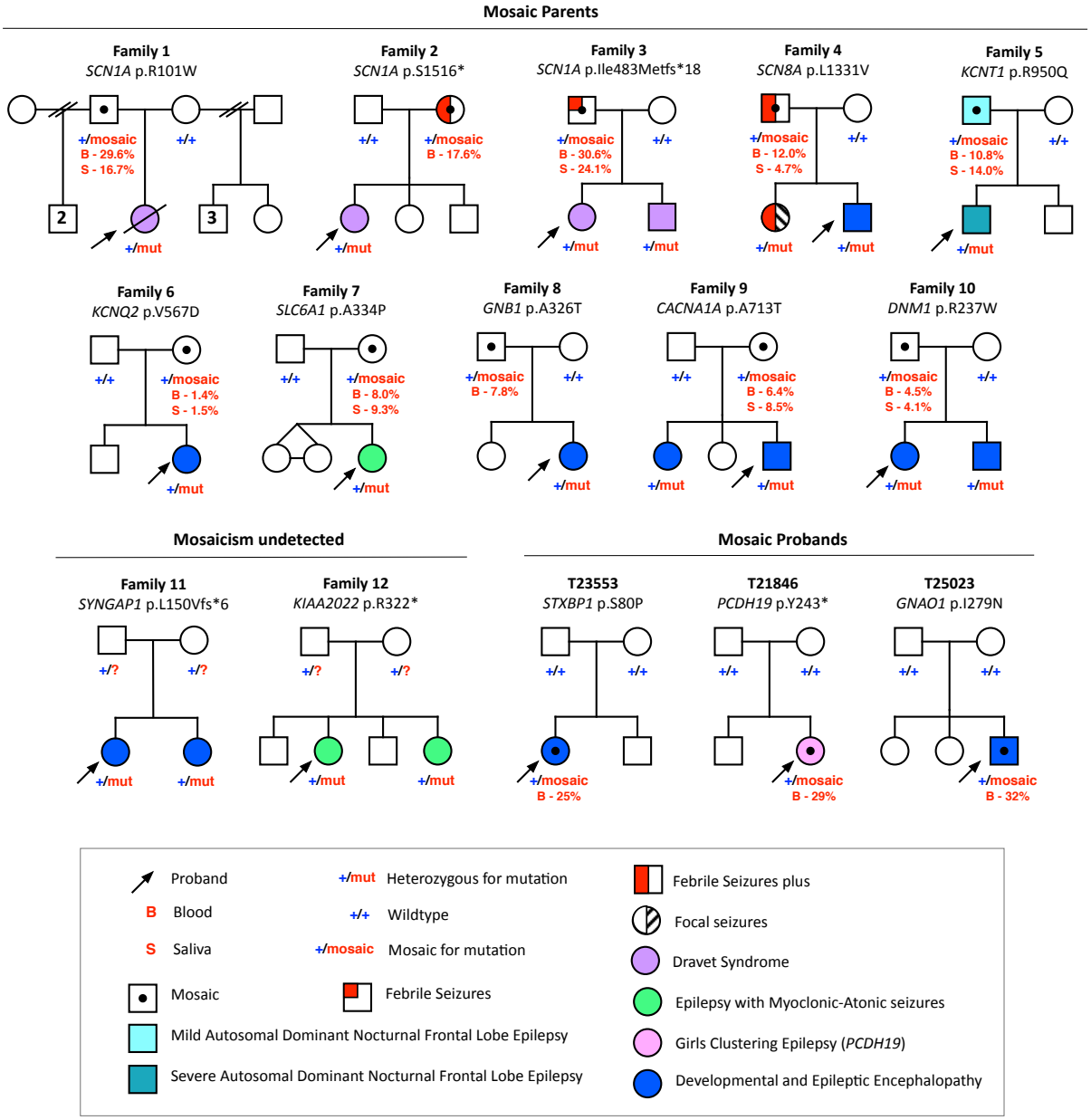

Figure S1. Pedigrees of families with confirmed (Family 1-10) or inferred (Family 11, 12) parental mosaicism or a mosaic proband.

Proband	Gene	Proband Phenotype	Transcript	cDNA Change	Protein Change	pAB	pCvg	pRef	pAlt	allCvg	allRef	Affected Sib?	Affected parent?
T23553	*STXBP1*	DEE	NM_003165.3	c.238T>C	p.S80P	0.25	72	54	18	119784	119332	No	No
T25023	*GNAO1*	DEE	NM_020988.2	c.836T>A	p.I279N	0.32	1175	799	373	53231	53131	No	No
T21846	*PCDH19*	DEE	NM_001184880.1	c.729C>A	p.Y243*	0.29	163	116	47	23921	23881	No	No

Table S1. Mosaic probands.
Legend: **DEE** – Developmental and Epileptic Encephalopathy, **pAB** - allele balance of the proband, **pCvg** - total coverage (unique captures) in the proband, **pRef** - the number of captures that support the reference allele in the proband, **pAlt** - the number of captures that support the alternate allele in the proband, **allCvg** - total captures using sequencing data from all individuals unrelated to the proband, used as the denominator to determine the variant-specific sequencing error rate, **allRef** - the number of captures that supported the reference call using sequencing data from all individuals unrelated to the proband

Proband	Mother	Father	Gene	Parental mosaicism?	Proband phenotype	Sibling phenotype	Sibling tested for pathogenic variant?	Concordant/ Discordant? Phenotype	Affected parent?
T25786	T25840	T25841	*SCN1A*	Paternal mosaicism. Family 3.	Dravet	Brother - Dravet	*SCN1A +ve*	**CONCORDANT**	Father - FS
T25417	T23749	T23736	*SYNGAP1*	Mosaicism not detected. Family 11.	DEE	Sister - DEE	*SYNGAP1 +ve*	**CONCORDANT**	No
T24107	T24108	T24109	*DNM1*	Paternal mosaicism. Family 10.	DEE	Brother - DEE	*DNM1 +ve*	**CONCORDANT**	No
T24139	T24628	T24627	*CACNA1A*	Maternal mosaicism. Family 9.	DEE	Sister - DEE	*CACNA1A +ve*	**CONCORDANT**	No
T990	T23797	T23796	*KIAA2022*	Mosaicism not detected. Family 12.	MAE	Sister - MAE	*KIAA2022 +ve*	**CONCORDANT**	No
T3929	T3931	T3930	*SCN8A*	Paternal mosaicism. Family 4.	DEE	Sister - FS+, focal seizures, learning difficulties	*SCN8A +ve*	**DISCORDANT**	Father - FS+
T21224	T25630	T25629	*KCNB1*	No	DEE	Brother - autistic features, no seizures, borderline intellect, frequent occipital spikes	*KCNB1 p.v. negative*	**DISCORDANT**	No
T38	8595	T37	*CHD2*	No	MAE	Sister - unconfirmed seizures	*CHD2 p.v. negative*	**DISCORDANT**	Mother - FS
T16910	T16912	T16909	*SCN1A*	No	Dravet	Sister - single FS	*SCN1A p.v. negative*	**DISCORDANT**	No

T888	T886	T887	*SCN1A*	No	Dravet	Sister - 2 x FS	*SCN1A p.v. negative*	**DISCORDANT**	No
T25001	T25273	T25274	*WDR45*	No	DEE	Maternal half sister - single FS	Not tested (no DNA)	**DISCORDANT**	No
T18436	T18437	T20202	*KCNH5*	No	DEE	Paternal half sister - FS	Not tested (no DNA)	**DISCORDANT**	Father - FS
T22888	T22889	T22890	*ARID1B*	No	DEE	Sister - single FS	Not tested (no DNA)	**DISCORDANT**	Mother - FS

Table S2. Families with more than one affected child.
Legend: **DEE** – Developmental and Epileptic Encephalopathy, **FS** – Febrile Seizure, **FS+** - Febrile Seizures Plus, **MAE** – Epilepsy with Myoclonic-Atonic Seizures, **p.v.** – pathogenic variant.

Proband	Mother	Father	Gene	Mosaicism detected?	Proband Phenotype	Affected parent?	Affected Sibling?
T3091	T3092	T3093	*SCN1A*	Maternal mosaicism. Family 2.	Dravet	Mother - FS	No
9092	T23573	9093	*KCNT1*	Paternal mosaicism. Family 5.	DEE (severe ADNFLE)	Father - Mild ADNFLE	No
T3929	T3931	T3930	*SCN8A*	Paternal mosaicism. Family 4.	DEE	Father - FS+	Sister - FS+, focal seizures, learning difficulties, SCN8A +ve
T25786	T25840	T25841	*SCN1A*	Paternal mosaicism. Family 3.	Dravet	Father - FS	Brother - Dravet syndrome, SCN1A +ve
T21156	T21502	T21501	*SLC2A1*	No	DEE	Mother - FS	No
T20340	T23374	T20341	*SCN2A*	No	DEE	Father - FS	No
T24322	T24641	T24640	*SCN2A*	No	DEE	Father - FS	No
31232	31234	31233	*SCN1A*	No	Dravet	Mother - FS	No
T3611	T3845	T3846	*SCN1A*	No	Dravet	Mother - unclassified childhood seizures	No
T882	T1169	T1168	*SCN1A*	No	Dravet	Father - FS	No
T21091	T21094	T21095	*SCN1A*	No	Dravet	Mother - 3 tonic-clonic seizures in adulthood	No
T23532	T23534	T23533	*GABRA1*	No	Dravet	Mother - FS	No
T25956	T25958	T25957	*CDKL5*	No	DEE	Mother - FLE	No
T18436	T18437	T20202	*KCNH5*	No	DEE	Father – FS	Paternal half sister - FS
T22888	T22889	T22890	*ARID1B*	No	DEE	Mother - FS	Sister - FS
T38	8595	T37	*CHD2*	No	MAE	Mother – FS	Sister - unconfirmed seizures

Table S3. Parents with a history of seizures.
Legend: **ADNFLE** – Autosomal Dominant Nocturnal Frontal Lobe Epilepsy, **DEE** – Developmental and Epileptic Encephalopathy, **FLE** – Frontal Lobe Epilepsy, **FS** – Febrile Seizure, **FS+** - Febrile Seizures Plus, **MAE** – Epilepsy with Myoclonic-Atonic Seizures.

Proband (Mosaic Family)	Proband phenotype	Genomic coordinates	Gene	GVS function	Proband AB	Mother Alt	Mother Cov	Father Alt	Father Cov	Error Rate
T25031 (#8)	DEE	chr1:1718817C>T	*GNB1*	missense	0.561	0	57	8	102	2E-04
T21006	WS	chr1:1735987T>C	*GNB1*	missense	0.579	0	409	1	1635	2E-04
T20555	IDEE	chr1:43393320A>G	*SLC2A1*	missense	0.484	0	615	0	519	4E-04
T18702	IDEE	chr1:43394680G>A	*SLC2A1*	missense	0.518	0	401	0	330	2E-04
T22667	DEE	chr1:43394876C>G	*SLC2A1*	splice-site	0.485	0	326	0	430	4E-04
e4693-1	EODEE	chr1:43395390C>G	*SLC2A1*	missense	0.468	0	405	0	520	1E-04
T21156	DEE	chr1:43396337A>C	*SLC2A1*	missense	0.444	0	204	0	230	8E-05
T21904	DEE	chr1:43396356G>A	*SLC2A1*	missense	0.460	1	245	0	211	4E-04
e4675-1	IDEE	chr1:43396798C>T	*SLC2A1*	stop-gain	0.455	2	491	0	818	1E-04
T26155	IDEE	chr1:111146515C>T	*KCNA2*	missense	0.512	2	493	1	316	4E-04
T1898	IDEE	chr2:149225978AT>A	*MBD5*	frameshift	0.483	0	381	0	389	1E-04
31144	EODEE	chr2:166170455T>C	*SCN2A*	missense	0.556	0	1229	0	1320	3E-04
e4699-1	IDEE	chr2:166201174T>C	*SCN2A*	missense	0.464	1	1030	4	1011	1E-03
31145	IDEE	chr2:166201201T>C	*SCN2A*	missense	0.519	0	260	0	319	3E-04
T22816	EIMFS	chr2:166201285T>G	*SCN2A*	missense	0.404	0	879	0	2862	9E-05
T23161	DEE	chr2:166210710C>A	*SCN2A*	missense	0.466	1	3691	1	2614	3E-04
T20340	DEE	chr2:166210838GA>G	*SCN2A*	frameshift	0.529	0	950	0	1251	2E-04
T24322	EODEE	chr2:166243481G>A	*SCN2A*	missense	0.544	0	1026	2	2141	2E-04
T24262	OS	chr2:166245217G>T	*SCN2A*	missense	0.473	2	1075	0	565	4E-04
T24127	EODEE	chr2:166245961G>A	*SCN2A*	missense	0.499	0	1039	0	576	3E-04
31232	Dravet	chr2:166848111G>A	*SCN1A*	stop-gain	0.515	0	919	0	550	1E-04
T3611	Dravet	chr2:166848129G>A	*SCN1A*	stop-gain	0.449	0	1484	0	1418	1E-04
T24455	Dravet	chr2:166848540C>T	*SCN1A*	missense	0.444	2	742	1	945	8E-04
T23445	Dravet	chr2:166850699TC>T	*SCN1A*	frameshift	0.522	0	2016	0	2743	3E-05
7962	Dravet	chr2:166850722G>A	*SCN1A*	missense	0.479	3	2607	0	1223	4E-04
T3091 (#2)	Dravet	chr2:166852557G>T	*SCN1A*	stop-gain	0.525	258	1468	0	1225	5E-04
T896	Dravet	chr2:166852557G>T	*SCN1A*	stop-gain	0.517	0	638	0	387	5E-04
T32753	Dravet	chr2:166854570TTATC>T	*SCN1A*	frameshift	0.575	0	457	0	600	0E+00
T19963	Dravet	chr2:166854571T>C	*SCN1A*	missense	0.460	0	3401	0	357	2E-04
T25786 (#3)	Dravet	chr2:166854574CT>C	*SCN1A*	frameshift	0.520	0	277	122	399	0E+00
T20264	Dravet	chr2:166854636A>G	*SCN1A*	missense	0.464	0	1019	0	1013	1E-04
T18466	Dravet	chr2:166859233G>A	*SCN1A*	missense	0.611	2	3753	1	3190	6E-04
T19875	DEE EAS	chr2:166866254G>A	*SCN1A*	missense	0.516	0	1409	0	1778	3E-04
T882	Dravet	chr2:166866255C>G	*SCN1A*	missense	0.443	0	1362	0	4991	4E-05
T16910	Dravet	chr2:166866262AG>G	*SCN1A*	frameshift	0.564	0	1483	0	1269	7E-06
T19196	Dravet	chr2:166866291AT>A	*SCN1A*	frameshift	0.502	0	1409	0	987	5E-05
T1144	Dravet	chr2:166892965C>A	*SCN1A*	stop-gain	0.494	0	204	0	259	4E-05
T21674	Dravet	chr2:166894396G>A	*SCN1A*	missense	0.539	0	418	0	266	4E-04

T2590	Dravet	chr2:166894401A>T	*SCN1A*	missense	0.466	0	444	0	414	1E-04
T19500	Dravet	chr2:166896094TC>T	*SCN1A*	frameshift	0.484	1	1721	0	1437	8E-05
T1832	Dravet	chr2:166897741C>T	*SCN1A*	splice-site	0.498	1	269	1	254	6E-04
T888	Dravet	chr2:166898935C>T	*SCN1A*	splice-site	0.523	0	292	0	292	3E-04
T18832	Dravet	chr2:166900484G>A	*SCN1A*	splice-site	0.482	0	1794	0	1482	2E-04
T1498	Dravet	chr2:166900497GA>G	*SCN1A*	frameshift	0.409	0	1555	0	995	1E-04
T1602	Dravet	chr2:166903392A>T	*SCN1A*	missense	0.462	0	1218	0	1354	3E-04
T2942	Dravet	chr2:166903460G>T	*SCN1A*	stop-gain	0.512	0	3003	0	2424	6E-04
T19076	DEE mild	chr2:166903476G>T	*SCN1A*	missense	0.473	0	2659	0	2698	4E-04
T22005	Dravet	chr2:166903479C>T	*SCN1A*	missense	0.486	1	1375	0	634	1E-04
T23245	Dravet	chr2:166903479C>G	*SCN1A*	missense	0.503	0	866	0	1675	7E-05
T26106	Dravet	chr2:166904244C>G	*SCN1A*	missense	0.455	0	900	0	532	8E-05
31792	Dravet	chr2:166908262C>A	*SCN1A*	stop-gain	0.485	0	301	0	247	6E-05
T21091	Dravet	chr2:166908266GAC>G	*SCN1A*	frameshift	0.516	0	940	0	1085	0E+00
T25634	EISE	chr2:166909379G>A	*SCN1A*	missense	0.492	1	1004	0	2323	5E-04
T32999	Dravet	chr2:166911245A>G	*SCN1A*	missense	0.495	0	656	0	871	2E-04
T1635	Dravet	chr2:166911254T>TGATTCAC	*SCN1A*	frameshift	0.539	0	614	0	421	0E+00
T25354	Dravet	chr2:166913012T>C	*SCN1A*	splice-site	0.479	3	1954	0	941	9E-04
T17775	Dravet	chr2:166915080G>T	*SCN1A*	stop-gain	0.415	0	334	0	332	3E-04
T20166	Dravet	chr2:166915161C>T	*SCN1A*	missense	0.551	1	573	0	614	7E-04
T25407 (#1)	Dravet	chr2:166915162G>A	*SCN1A*	missense	0.477	0	259	5	30	2E-04
8707	Dravet	chr2:166930020GG>G	*SCN1A*	frameshift	0.520	0	2464	0	1160	0E+00
T23870	IDEE	chr2:197081742C>T	*HECW2*	missense	0.480	0	2289	0	689	4E-04
e4914-1	MAE	chr3:11067229T>C	*SLC6A1*	missense	0.484	0	279	0	364	6E-04
e4919-1	MAE	chr3:11067457A>G	*SLC6A1*	splice-site	0.421	0	968	0	474	1E-04
T21866 (#7)	MAE	chr3:11067967G>C	*SLC6A1*	missense	0.506	246	3056	0	2694	5E-04
T23549	DEE	chr5:88100558A>G	*MEF2C*	missense	0.433	0	1215	0	516	4E-04
T21213	DEE	chr5:160758056G>A	*GABRB2*	missense	0.469	0	1256	0	1391	3E-04
T23211	EODEE	chr5:160761861A>G	*GABRB2*	missense	0.503	0	1281	0	352	6E-04
T23532	Dravet	chr5:161300202G>A	*GABRA1*	missense	0.484	3	2379	0	2397	1E-03
e4738-1	DEE mild	chr5:161569293A>C	*GABRG2*	missense	0.647	0	284	0	342	1E-04
T20719	MAE	chr5:161576159G>A	*GABRG2*	missense	0.430	0	305	1	466	1E-03
T25417 (#11)	DEE	chr6:33400506G >GAAGTCACAACCCA	*SYNGAP1*	frameshift	0.462	0	834	0	1351	0E+00
T15923	DEE	chr6:33405482G>A	*SYNGAP1*	stop-gain	0.511	0	1046	0	836	5E-04
T22888	DEE	chr6:157528565AC>A	*ARID1B*	frameshift	0.482	0	217	0	506	4E-05
e4682-1	EODEE	chr9:130416000G>GT	*STXBP1*	frameshift	0.502	0	835	0	2862	5E-06
T20627	DEE	chr9:130422380CACTG>C	*STXBP1*	frameshift	0.502	0	395	0	980	2E-05
T23930	EODEE	chr9:130423407CT>C	*STXBP1*	frameshift	0.511	0	697	0	336	9E-06
T23122	EODEE	chr9:130425622C>T	*STXBP1*	missense	0.499	2	2380	1	2652	6E-04
T1915	Dravet	chr9:130430411G>A	*STXBP1*	missense	0.400	0	323	0	934	6E-04

T22856	EODEE	chr9:130435490T>C	*STXBP1*	missense	0.473	2	1003	0	935	6E-04
T23289	IDEE	chr9:130446652A>G	*STXBP1*	missense	0.453	0	725	0	1183	3E-04
T24107 (#10)	DEE	chr9:130982480C>T	*DNM1*	missense	0.709	0	2611	118	2623	2E-04
9092 (#5)	DEE (ADNFLE)	chr9:138675877G>A	*KCNT1*	missense	0.501	0	25	21	195	1E-03
T23159	EME	chr11:35336626A>G	*SLC1A2*	missense	0.477	2	292	1	332	5E-04
T3129	DEE CSWS	chr12:13828685C>T	*GRIN2B*	stop-gain	0.478	5	1671	2	2260	2E-03
T3929 (#4)	DEE	chr12:52180374C>G	*SCN8A*	missense	0.553	0	401	119	994	0E+00
T23111	EODEE	chr12:52200884C>T	*SCN8A*	missense	0.528	2	1345	0	1655	4E-04
T18436	DEE EAS	chr14:63417240C>T	*KCNH5*	missense	0.462	0	1014	0	835	1E-03
T23296	MAE	chr14:102514979C>A	*DYNC1H1*	missense	0.422	0	894	0	396	9E-05
T25708	LGS	chr15:26806246C>T	*GABRB3*	missense	0.504	0	1123	0	494	3E-04
T25111	DEE	chr15:26812818G>T	*GABRB3*	missense	0.450	0	495	0	477	2E-03
T22598	IDEE	chr15:26825603T>A	*GABRB3*	missense	0.519	7	2366	6	2401	3E-03
T18697	DEE	chr15:93470540C>T	*CHD2*	stop-gain	0.406	1	792	0	881	9E-04
T2608	LGS	chr15:93492254C>CT	*CHD2*	frameshift	0.447	0	1757	0	1697	0E+00
T22432	DEE	chr15:93492257C>T	*CHD2*	stop-gain	0.394	2	1496	1	1812	5E-04
T17756	DEE	chr15:93496726T>C	*CHD2*	missense	0.464	4	1896	1	2140	8E-04
T18431	DEE	chr15:93515610T>C	*CHD2*	missense	0.456	0	268	2	1358	7E-04
T38	MAE	chr15:93545499CAAAG>C	*CHD2*	frameshift	0.413	0	4281	0	4487	3E-03
T24220	DEE	chr15:93557952T>TG	*CHD2*	frameshift	0.447	0	1238	0	1542	1E-05
T20240	MAE	chr15:93563264AAG>A	*CHD2*	frameshift	0.500	0	910	0	911	0E+00
T25816	EODEE	chr16:56370729C>T	*GNAO1*	missense	0.523	0	352	0	329	1E-03
T23039	EODEE	chr19:13414398C>T	*CACNA1A*	missense	0.430	0	903	0	233	2E-04
T24139 (#9)	EODEE	chr19:13414398C>T	*CACNA1A*	missense	0.470	31	481	0	318	2E-04
T21224	IDEE	chr20:47990896C>T	*KCNB1*	missense	0.479	0	995	0	951	1E-04
T26654	IDEE	chr20:47990953C>T	*KCNB1*	missense	0.522	1	2490	0	3021	1E-04
T25029	OS -> LGS	chr20:62044824C>T	*KCNQ2*	missense	0.429	2	450	0	502	2E-03
T23926 (#6)	OS	chr20:62044866A>T	*KCNQ2*	missense	0.519	4	284	0	267	2E-03
T21758	OS	chr20:62070028T>C	*KCNQ2*	missense	0.472	0	816	0	413	5E-04
T26147	WS + FCD	chr22:32234705CA>C	*DEPDC5*	frameshift	0.482	0	242	0	224	9E-05
T25956	IDEE	chrX:18598088G>C	*CDKL5*	missense	0.492	0	748	0	548	2E-04
T20819	EODEE	chrX:18602381A>G	*CDKL5*	splice-site	0.994	1	1235	2	2151	8E-04
e4702-1	EODEE	chrX:18602432C>G	*CDKL5*	stop-gain	0.517	0	597	0	1057	6E-05
T22954	EODEE	chrX:18602464T>C	*CDKL5*	missense	0.502	1	1639	0	996	5E-04
T24729	LGS	chrX:48932539CAT>C	*WDR45*	frameshift	0.524	0	573	0	211	0E+00
T25001	DEE	chrX:48933232G>A	*WDR45*	stop-gain	0.489	0	639	0	295	7E-05
T20993	WS -> LGS	chrX:48933304AG>A	*WDR45*	frameshift	0.525	0	381	0	1979	1E-05
e4695-1	DEE	chrX:48933318C>T	*WDR45*	missense	0.512	0	1558	0	432	1E-04
T22244	DEE	chrX:73962417G>A	*KIAA2022*	stop-gain	0.643	1	459	1	310	1E-03
T990 (#12)	MAE	chrX:73963428G>A	*KIAA2022*	stop-gain	0.497	1	570	1	201	1E-03
T1828	GCE	chrX:99662770A>G	*PCDH19*	missense	0.483	0	302	0	239	6E-04

T22482	GCE	chrX:99663098G>GT	*PCDH19*	frameshift	0.521	0	303	0	322	0E+00

Table S4. Sequencing data, clinical and molecular details for all 120 families*.

Legend: **AB** – allele balance, **ADNFLE** – Autosomal Dominant Nocturnal Frontal Lobe Epilepsy, **AHC** – Alternating Hemiplegia of Childhood, **Alt** – the number of captures that support the alternate allele, **Cov** – total coverage (unique captures by single molecule tagging), **CSWS** – Continuous Spike and Wave during Slow-wave Sleep, **DEE** – Developmental and Epieptic Encephalopathy (not otherwise specified), **EAS** – Epilepsy-aphasia syndrome, **EIMFS** – Epilepsy of Infancy with Migrating Focal Seizures, **EISE** – Early Infantile SCN1A Encephalopathy, **EOAE** – Early Onset Absence Epilepsy, **EODEE** – Early Onset Developmental and Epileptic Encephalopathy, **FCD** – Focal Cortical Dysplasia, **FLE** – Frontal Lobe Epilepsy, **GCE** – Girls Clustering Epilepsy (PCDH19), **IDEE** – Infantile Developmental and Epileptic Encephalopathy, **LGS** – Lennox Gastaut Syndrome, **MAE** – Epilepsy with Myoclonic-Atonic seizures, **OS** – Ohtahara syndrome, **WS** – West syndrome, **->** – evolving to.

* An expanded version of this table in excel format is available upon request

ADDITIONAL REFERENCES

1. Carvill GL, Heavin SB, Yendle SC, et al. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in CHD2 and SYNGAP1. Nature Genetics. 2013;45:825-30.
2. Carvill GL, McMahon JM, Schneider A, et al. Mutations in the GABA Transporter SLC6A1 Cause Epilepsy with Myoclonic-Atonic Seizures. American Journal of Human Genetics. 2015;96:808-15.
3. Epi4K Consortium. De Novo Mutations in SLC1A2 and CACNA1A Are Important Causes of Epileptic Encephalopathies. American Journal of Human Genetics. 2016;99:287-98.
4. Reutens DC, Howell RA, Gebert KE, Berkovic SF. Validation of a questionnaire for clinical seizure diagnosis. Epilepsia. 1992;33:1065-71.
5. Hiatt JB, Pritchard CC, Salipante SJ, O'Roak BJ, Shendure J. Single molecule molecular inversion probes for targeted, high-accuracy detection of low-frequency variation. Genome Research. 2013;23:843-54.
6. Stessman HA, Xiong B, Coe BP, et al. Targeted sequencing identifies 91 neurodevelopmental-disorder risk genes with autism and developmental-disability biases. Nature Genetics. 2017;49:515-26.
7. O'Roak BJ, Vives L, Fu W, et al. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. Science. 2012;338:1619-22.
8. Zhang J, Kobert K, Flouri T, Stamatakis A. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics. 2014;30:614-20.
9. Borsting C, Sanchez JJ, Hansen HE, Hansen AJ, Bruun HQ, Morling N. Performance of the SNPforID 52 SNP-plex assay in paternity testing. Forensic Science International Genetics. 2008;2:292-300.