Molecular diagnosis of patients with epilepsy and developmental delay using a customized panel of epilepsy genes

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Abstract

Pediatric epilepsies are a group of disorders with a broad phenotypic spectrum that are associated with great genetic heterogeneity, thus making sequential single-gene testing an impractical basis for diagnostic strategy. The advent of next-generation sequencing has increased the success rate of epilepsy diagnosis, and targeted resequencing using genetic panels is the most cost-effective choice. We report the results found in a group of 87 patients with epilepsy and developmental delay using targeted next generation sequencing (custom-designed Haloplex panel). Using this gene panel, we were able to identify disease-causing variants in 17 out of 87 (19.5%) analyzed patients, all found in known epilepsy-associated genes (KCNQ2, CDKL5, STXBP1, SCN1A, PCDH19, POLG, SLC2A1, ARX, ALG13, CHD2, SYNGAP1, and GRIN1). Twelve of 18 variants arose de novo and 6 were novel. The highest yield was found in patients with onset in the first years of life, especially in patients classified as having early-onset epileptic encephalopathy. Knowledge of the underlying genetic cause provides essential information on prognosis and could be used to avoid unnecessary studies, which may result in a greater diagnostic cost-effectiveness.

Introduction

Epilepsy is a common neurologic disorder in childhood, with a prevalence of 300–600 per 100000. About 30% of children with epilepsy present behavioral or cognitive impairment [1]. Among the most severe forms of childhood epilepsy are the so-called epileptic encephalopathies (EEs), which include a number of heterogeneous early-onset clinical disorders characterised by refractory seizures, developmental delay, or regression associated with ongoing epileptic activity, and poor prognosis in the majority of the patients [2, 3]. Dravet, Ohtahara,
and West syndromes are some of the most common EEs [2]; however, many neonates and infants with EEs do not fit into any of the proposed epileptic syndromes [4].

With the advancement of technologies for genetic diagnosis, genetic defects have been increasingly recognised as causes of different types of pediatric epilepsies, and also seem to account for a significant number of EEs [5, 6]. Genes involved in ion channelopathies, neuronal transmission, brain development, or synaptic functions have been reported to be associated with EE [7]. To date, more than 500 genes have been linked to epilepsy, and several genes—including STXBP1, ARX, SLC25A22, KCNQ2, CDKL5, SCN1A, and PCDH19—have been found to be associated with EEs [8–11]. The genetic and phenotypic heterogeneity in pediatric epilepsies [12–20] coupled with the fact that very few cases are explained by mutations in the same gene [21] make sequential single-gene testing impractical. Genetic testing panels open new possibilities for the diagnosis of this type of epilepsies, especially those for which diagnosis is otherwise unclear [8, 22–27].

The aim of this study was to perform a comprehensive genetic analysis using next-generation sequencing (NGS) technology to analyze more than 80 genes previously associated with epilepsy in 87 patients with epilepsy and developmental delay.

**Materials and methods**

**Patients**

We selected 87 patients with epilepsy and developmental delay of unexplained origin referred to our laboratory for genetic study. Most patients had been previously studied in order to rule out a structural or metabolic etiology. Patients' medical histories and results from laboratory testing were obtained by face-to-face interview and by consulting medical records. When available, information regarding the patient’s clinical, imaging, and neurophysiology data were reviewed by 2 epileptologists with experience in clinical epilepsy genetics, and phenotypes were classified into known electroclinical syndromes. When there was insufficient information, or the phenotype did not correspond to any recognizable syndrome, patients were included in the unclassified group. Before performing the genetic panel, 41 (47.1%) patients had been studied for mutations in selected epilepsy genes with conventional techniques (see Table 1).

Informed parental consent for genetic testing was obtained in all cases. DNA samples were extracted from peripheral blood lymphocytes using standard procedures. The study was approved by the local ethics committee (Hospital Universitario Fundación Jiménez Díaz).

**Epilepsy panel**

We designed 2 panels using Agilent’s SureDesign tool (www.agilent.com/genomics/suredesign) including genes that were known to be involved in epilepsy as a phenotypic feature according to the Online Mendelian Inheritance in Man (OMIM) database (http://www.ncbi.nlm.nih.gov/omim). These genetic panels cover exonic regions as well as exon–intron boundaries of the selected genes.

We designed a first panel comprising 83 genes (S1 Table) to screen a cohort of 44 patients. A second panel was designed with 106 genes (S2 Table) to screen 43 more patients. This second panel included new genes that have been more recently associated with epilepsy, excluding some genes contained in the first panel.

Theoretically, panel 1 included 17612 amplicons covering 873721 Mbp (99.42% of the region of interest), and panel 2 included 19597 amplicons, 1105 Mbp and 99.78%. Certain regions did not achieve a satisfying coverage and needed resequencing by Sanger.
| ID | Sex | Phenotype   | Age at seizure onset | Previous genetic analysis | Gene / Transcript | Variant | dbSNP147 / MAF | Inheritance | PolyPhen2 / SIFT (score) | GERP (score) | ExAC (Allele frequency) |
|----|-----|-------------|----------------------|--------------------------|-------------------|---------|----------------|-------------|------------------------|-------------|------------------------|
| 1  | F   | EOEE        | 2 days               | PNPO STXBP1              | KCNQ2 / NM_004518.5 | c.602G>A / p. Arg201His | Reported by Carvill et al., 2013 | IVF         | 0.979 / 0              | 3.88         | Not present            |
| 2  | F   | EOEE        | NA                   | None                     | None              |          |                |             | IVF                     | 0.979 / 0   | Not present            |
| 3  | M   | EOEE        | 17 hours             | None                     | None              |          |                |             | De novo                 | 2.84        | Not present            |
| 4  | M   | Unclassified EE | 24 hours             | None                     | STXBP1 / NM_00103221.3 | c.1216C>T / p. Arg406Cys | rs796053367 / NA | De novo   | 0.053 / 0.03           | 3.38         | Not present            |
| 5  | F   | EOEE        | 20 days              | KCNQ2 PRRT2 SCN2A        | ALG13 / NM_001039210.4 | c.320A>G / p. Asn107Ser | rs398122394 / NA | De novo   | 0.869 / 0              | 2.13         | Not present            |
| 6  | F   | NLES        | 5 months             | ARX CDKL5 SCN1A          | SCN1A / NM_001105243.1 | c.698A>G / p. Asp233Gly | Not reported | Paternally inherited | 0.999 / 0 | 6.08 | Not present |
| 7  | M   | Unclassified EE | 1 months             | KCNQ2 CDKL5 SCN1A        | SCN1A / NM_001165963.1 | c.52_53insT / p. Val19CysfsTer3 | Not reported | De novo | NA | 5.56 | Not present |
| 8  | F   | Unclassified EE | 6 months             | None                     | CHD2 / NM_001042572.2 | c.2317G>A / p. Cys773Tyr | Reported by Fehr et al., 2015 | De novo | 0.998 / 0 | 5.98 | Not present |
| 9  | F   | Unclassified EE | 1 months             | None                     | PRRT2 / NM_001037343.1 | c.533G>A / p. Arg178Gln | rs267606715 / NA | De novo | 1 / 0 | 5.60 | Not present |
| 10 | F   | SMEI        | 6 months             | None                     | PRRT2 / NM_00101105243.1 | c.602G>A / p. Arg201His | Not reported | De novo | NA | 5.24 | Not present |
| 11 | F   | Unclassified EE | 4 months             | PCDH19 SCN1A              | SCN1A / NM_001165963.1 | c.602G>A / p. Val19CysfsTer3 | rs79472827 / NA | De novo | NA | 5.24 | Not present |
| 12 | M   | Unclassified EE | 6 months             | SLC2A1 SynGAP1            | CHD2 / NM_001042572.2 | c.2317G>A / p. Glu773Lys | Not reported | Parents not available | 0.019 / 0.28 | 5.18 | Not present |
| 13 | F   | Unclassified EE | 2 months             | SLC2A1 SLC2A2             | SLC2A1 / NM_001042572.2 | c.115G>A / p. Val19CysfsTer3 | Not reported | De novo | NA | 5.24 | Not present |
| 14 | M   | Unclassified EE | 18 months            | SCN1A SynGAP1             | SCN1A / NM_001190366.1 | c.333_334insG / p. Lys114GlufsTer38 | Not reported | De novo | NA | -2.27 | Not present |
| 15 | M   | EOEE        | 1 months             | None                     | ARX / NM_139058.2 | c.196G>A / p. Gly66Ser | rs105751856 / NA | De novo | 0.788 / 0.21 | 4.90 | Not present |
| 16 | M   | Unclassified EE | 3 years              | SRPX2                    | POLG / NM_001126131.1 | c.156_158dupGCA / p. Gin5dup | rs41550117 / NA | Maternally inherited | NA | 0.00 | 0.01921 |
| 17 | F   | Unclassified EE | 6 months             | CDKL5 FOXG1              | GRIN1 / NM_0008326.2 | c.2504C>A / p. Ala835Asp | rs41549716 / 0.02 | Parents not available | 0.948 / 0.7 | 1.47 | 0.006277 |

F: female, M: male, EOEE: early-onset epileptic encephalopathy, EE: epileptic encephalopathy, NLES: Non-lesional epileptic spasms, SMEI: severe myoclonic epilepsy of infancy, dbSNP: single nucleotide polymorphism database, MAF: minor allele frequency, NA: not available, IVF: in vitro fertilisation, PolyPhen2: polymorphism phenotyping version 2, SIFT: sorting intolerant from tolerant, GERP: genomic evolutionary rate profiling, ExAC: exome aggregation consortium.

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Target enrichment method

We used customized in-solution target enrichment followed by NGS to screen for variants in our 2 cohorts of patients. A library of all coding exons and intron-exon boundaries was prepared using a HaloPlex target enrichment kit (Agilent, Santa Clara, USA) following the manufacturer’s instructions. Briefly, we fragmented the human genome (the samples were digested by 16 different restriction enzymes to create a library of gDNA restriction fragments) and enriched for the coding regions of genes by using complementary highly specific biotinylated probes. HaloPlex probes are designed to hybridise selectively to fragments originating from target regions of the genome. and to direct circularisation of the targeted DNA fragments. Hybridized probes were captured with magnetic beads and target fragments were ligated to create circular DNA molecules. Subsequently, libraries were amplified by PCR, introducing unique index sequences that allow all pools to be sequenced together. Sequencing was performed using the NGS MiSeq Illumina sequencer (Illumina, Inc.). As an acceptance threshold value we selected a Q-score of 30, corresponding to a 1:1000 error rate.

Bioinformatics tools

Fastq files from the sequencer were redirected to a custom pipeline for HaloPlex™ Target Enrichment System on the DNA nexus platform and/or to Agilent Surecall software. Briefly, reads were aligned to the human reference genome (GRCh37/hg19) (http://hgdownload.cse.ucsc.edu/) with Burrows-Wheeler Aligner (BWA) [28] and variants were called using at least 2 of the 3 following variant callers: Genome Analysis Toolkit (GATK) [29–31], Freebayes [32] (both within the DNA nexus platform), and Base Alignment Quality (BAQ) Single Nucleotide Polymorphism (SNP) caller (within SureCall tool).

Variants passing quality filters were annotated separately against NCBI RefGene (http://www.ncbi.nlm.nih.gov) and ENSEMBL Variant Effect Predictor ver.72 (http://www.ensembl.org/info/docs/tools/vep).

Prioritization of candidate genes

Variants were further filtered out to exclude all variants classified as synonymous, non-pathogenic, or with a frequency above 0.01 in control populations (data from dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/), 1000 Genomes Project (http://1000genomes.org), Exome Sequencing Project (http://evs.gs.washington.edu/EVS/), and Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org/)). We attempted to estimate the putative pathogenic effect of non-reported suspected variants with conventional and freely available online tools, such as Polymorphism Phenotyping version 2 (Polyphen2) (http://genetics.bwh.harvard.edu/pph2/), Sorting Intolerant From Tolerant (SIFT) (http://sift.bii.a-star.edu.sg/), and HSF (http://www.umdb.udb.edu/HSF/) [33–35]. PolyPhen2 scores of less than 0.15 are predicted to be benign, scores from 0.15 to 0.85 as possibly damaging, and scores greater than 0.85 are interpreted as probably damaging. SIFT scores of less than 0.05 are predicted to be deleterious and those greater than or equal to 0.05 are predicted to be tolerated. Also, we used Exomiser (http://www.sanger.ac.uk/resources/databases/exomiser/) [36], an online tool that functionally annotates and prioritises mutated genes using variant frequency, predicted pathogenicity, inheritance pattern, and model organism phenotype data as criteria. Scores are based on Mutation Taster [35], SIFT [37], Polyphen2 [34], and GERP [38]. GERP scores ranged from -12.3 to 6.17, with 6.17 being the most highly conserved.

Finally, the selected variants were evaluated within the context of their individual phenotype and clinical data. Putatively causative mutations were validated by conventional Sanger sequencing and tested by segregation analysis when possible.
Criteria for pathogenicity

We classified a novel variant as pathogenic according to the international guidelines of the American College of Medical Genetics (ACMG) Laboratory Practice Committee Working Group [39].

Results

We recruited, studied, and classified 87 patients with epilepsy mostly with an onset in the first year of life (68/87, 78.2%), and with developmental delay. Clinical diagnoses are summarized in Table 2. A large proportion of patients were unable to be classified, mainly due to incomplete clinical data (56/87). The patients were analyzed using a targeted next-generation custom gene panel. A mean coverage of 263× was obtained per sample (minimum 83× and maximum 443×), with 88% of bases covered at more than 30×. The percentage of read mapped to the reference genome was between 84.8% and 91.8%, with a mean of 88.3%.

After a stringent filtering procedure was carried out, a total of 18 presumed disease-causing variants in 12 genes were detected, including KCNQ2 (n = 4), CDKL5 (n = 3), SCN1A (n = 1), PCDH19 (n = 1), STXBPI (n = 1), SLC2A1 (n = 1), ARX (n = 1), ALG13 (n = 1), SYNGAP1 (n = 1), GRIN1 (n = 1), CHD2 (n = 1), and POLG (n = 2). We identified 3 (16.7%) frame-shift insertion-deletion, 2 (11.1%) putative splice site, and 13 (72.2%) missense variants, of which 12 (66.7%) arose de novo and 6 (33.3%) were novel.

Genomic evolutionary rate profiling (GERP) score showed that these variants affected highly conserved amino acids in mammals and were reported to be deleterious in the prediction programs used. In total, we were able to identify 18 disease-causing variants in 17 patients.

Of the 17/87 (19.5%) patients with positive findings, 10/44 (22.7%) had unclassified EEs, 5/9 (55.6%) had EOEE, 1/1 (100%) was diagnosed with severe myoclonic epilepsy of infancy (SMEI), and 1/12 (8.3%) was included in NLES group.

An overview of all detected variants is shown in Table 1.

It is worth noting that the positive cases in our panel had previously undergone different clinical and genetic tests, including karyotyping (41.2%, (7/17)), magnetic resonance imaging (MRI) (100%, (17/17)), metabolic screening (70.6%, (12/17)), mitochondrial DNA screening (23.5% (4/17)), comparative genomic hybridisation (CGH) array test (5.9% (1/17)), and sequential single-gene analysis (1–6 genes, see Table 1) (58.8% (10/17)).

Four point variants in KCNQ2 were identified in 3 patients with EOEE and one patient with unclassified EE: c.602G>A (patients 1 and 2), c.601C>T (patient 3), and c.803T>C

| Clinical diagnoses                              | n  | %    |
|-----------------------------------------------|----|------|
| Non-lesional epileptic spasms (NLES)          | 12 | 13.8%|
| Early-onset epileptic encephalopathy (EOEE)   | 9  | 10.3%|
| Landau-Kleffner syndrome (LKS)                | 1  | 1.1% |
| Severe myoclonic epilepsy in infancy (SMEI)    | 1  | 1.1% |
| Myoclonic-astatic epilepsy (MAE)              | 1  | 1.1% |
| Unclassified epileptic syndromes              | 44 | 50.6%|
| Epileptic encephalopathies (EEs)              | 8  | 9.2% |
| Generalized epilepsies                        | 4  | 4.6% |

*Early-onset epileptic encephalopathy includes Ohtahara syndrome (OS) and early myoclonic encephalopathy (EME).
Two variants arose de novo (see Table 1). Parental DNA samples (either parent) were not available for patient 3, or no paternal DNA sample was available for patient 1 (both were born following in vitro fertilisation (IVF)).

Interestingly, a variant affecting the same codon as in patients 1 and 2 has been reported, though with a different base substitution (Arg>Cys) [40].

The variants c.601C>T and c.602G>A are located in the transmembrane S4 domain, and the c.803T>C variant is located in the pore-forming H5 domain of the protein. All 3 variants are predicted to be pathogenic.

We also detected a previously reported STXBP1 heterozygous variant (c.1216C>T, rs796053367) in a patient diagnosed as having EOEE. This causative variant is located at exon 14, leading to the substitution of a conserved residue, R406, in domain 3b of STXBP1, and was not found in her parents [41, 42]. All patients described above had onset in the first 3 weeks of life and the electroencephalogram (EEG) showed a burst-suppression pattern.

Three de novo CDKL5 variants (c.52_53insT, c.377G>A, and rs267606715) presumed to be disease-causing were identified in 3 patients belonging to the unclassified EE group (see Table 1). The variants c.52_53insT and c.377G>A are located in the catalytic domain of the protein. Furthermore, the c.52_53insT variant affects the ATP-binding site and produces a truncated protein [43].

We have also identified pathogenic variants in ALG13, GRIN1, ARX, SCN1A, PCDH19, SLC2A1, CHD2, SYNGAP1, and POLG (see Table 1).

The variant found in ALG13 (c.320A>G) in a patient with NLES who progressed to Lennox-Gastaut syndrome (LGS) has been previously reported (rs398122394) as pathogenic and is located in the region where glycosyltransferase activity resides.

Two causative de novo variants in GRIN1 (c.2504C>A, p.Ala835Asp) and ARX (c.196G>A, p.Gly66Ser, rs1057518564) were found in a patient with an unclassified EE (patient 17) and in another diagnosed as EOEE (patient 15), respectively. The GRIN1 variant affects the calmodulin-binding domain, a highly conserved domain of the N-methyl-D-aspartate (NMDA)-receptor 1 [44]. Variants in this domain disturb interactions with intracellular proteins, which may impair receptor function [45].

We identified a de novo SCN1A splicing variant (c.115-2A>G, rs794726827) in a patient with unclassified EE. This pathogenic variant affects the splice-donor site in intron 4 and is located in the S3 transmembrane segment of domain I of the SCN1A protein. Human Splicing Finder (HSF) does not predict a cryptic splice-site activation; therefore, this variant may lead to skipping of exon 4, resulting in an affected channel. We could not confirm the predicted consequences because RNA samples were not available.

A paternally inherited PCDH19 variant (c.698A>G, p.Asp233Gly) was detected in one female diagnosed with SMEI. This novel variant is located in the first exon, which codifies the extracellular domain of the protein. The patient did not present any autistic features and her father was asymptomatic, contrasting with data reported by other authors [46, 47].

Patient 13 showed a heterozygous splice-site pathogenic variant (c.115-2A>G) in SLC2A1, which was confirmed as de novo. This variant affects the splice-acceptor site of the third exon but, according to the HSF, a cryptic splice site is activated. It causes a variation in the length of the exon, eliminating 9 nucleotides, which results in a loss of 3 amino acids in the protein. We could not test the functional consequence of this splice-site variant because the RNA samples were not available.

A G>A transition in the nucleotide 2317 in CHD2, which produces a Glu>Lys substitution in position 337, was found in patient 12, who had an unclassified EE. This change is located in the first chromodomain of the protein, likely affecting the remodeling of chromatin.
A novel disease-causing variant in SYNGAP1 (c.333_334insG, p.Lys114GlufsTer38) was found in a patient diagnosed as unclassified EE. This variant was not found in the parents. The variant p.Lys114GlufsTer38 is located in the pleckstrin homology domain in the N-terminal segment of the protein, and generates a truncated protein.

Finally, we identified 2 reported pathogenic variants in POLG (c.156_158dupGCA, p. Gln52dup; c.2492A>G, p.Tyr831Cys) in a patient included as an unclassified EE. The first variant was inherited from his mother, though the heritability of the second mutation could not be confirmed. The patient presented a late-onset unclassified EE with posterior electrical and neuroimaging abnormalities compatible with those previously described in patients harboring variants in POLG.

Discussion

NGS panels are now used widely in the clinical setting to identify genetic causes of epilepsy, replacing the traditional gene-by-gene approach. The genetic heterogeneity and the phenotypic overlap in severe epilepsies beginning in infancy and early childhood make multigene panel analysis a useful diagnostic tool.

Results from recent large studies incorporating NGS of patients with EE reveal that up to 30% of cases can be conclusively resolved with current technologies [48].

In this study, we describe the development of a Haloplex-based NGS assay in 87 patients with epilepsy and developmental delay. Applying this gene panel analysis, we were able to identify deleterious variants in 19.5% patients (17 of 87). Our results are in accordance with those previously reported by other authors, with diagnostic yields ranging between 10% and 48.5% [8, 23, 26, 27, 49–55]. Recent data show that de novo variants play an important role in EEs [56–58]. In our study, 12 out of 18 (66.7%) pathogenic variants were shown to be de novo.

We identified positive findings in most known prominent epilepsy genes such as KCNQ2 [59], CDKL5 [60], STXBP1 [61], SCN1A [62, 63], PCDH19 [64], POLG [65], SLC2A1 [66], and ARX [67] and in others more recently associated with EE such as ALGI3 [56], CHD2 [68], SYNGAP1 [69], and GRINI [70].

All of these genes are well established for severe pediatric epilepsies. We found a causative variant in 10 of 44 patients diagnosed with unclassified EE, the majority (8 of 10) with seizure onset in the first years of life, and 5 of 9 classified as EOEE. The overall positive rates were 14.3% and 55.6% in these groups of patients, respectively [53, 71, 72].

As mentioned above, the positive findings were related to genes well established as being causative of severe epilepsies beginning in infancy and early childhood and are consistent with the phenotypes of our patients, although the genotype could have been unsuspected.

In our panel analysis, we were unable to detect causative variants in 70 out of the 87 patients belonging to the NLES group, LGS, Landau-Kleffner syndrome (LKS), myoclonic-astatic epilepsy (MAE), and malignant migrating partial seizures of infancy (MMPSI) groups categorized as EEs, and in groups consisting of unclassified generalized and focal epilepsies. There are several reasons for these negative results. First, the patients were recruited for research purposes. Subsequent clinical and genetic studies identified the etiology in 12 cases: 9 patients showed structural brain abnormalities on MRI scan, 2 individuals carried a mitochondrial pathogenic variant, and 1 patient harboured a heterozygous deletion in PCDH19 that was not detected in the panel. The finding of a lesion, previously not detected, is not infrequent in the pediatric population mostly because of the difficulty to identify focal cortical dysplasia in late infancy and early childhood but also due to the increasing use of higher field MRI. Second, as we did not have detailed phenotypic data for 20 patients and many of these patients were studied at early stages of the disease, the final diagnosis may have been modified (in fact, during follow-
up 1 patient was finally diagnosed with Jeavons syndrome). Some of these patients were recruited a substantial amount of time ago and it is likely that other clinical or genetic tests could shed light on the underlying etiology (e.g. a CGH-array in children with epilepsy associated to ID with/without dysmorphic features). Finally, the absence of any presumed disease-causing variant in 37 patients with intensive follow-up and without relevant clinical changes was probably due to the fact that the causative gene was not present in our design. On the other hand, it should be noted that the findings in the negative cases included in epileptic disorders with a low diagnostic yield are in accordance with data reported by other authors [8, 17, 23, 25, 49–54].

Our study confirms the last published findings reported by other authors [26, 53] regarding the diagnostic yield of genetic testing in patients with severe pediatric epilepsies (especially in patients with early-onset epileptic encephalopathies). Additionally, considering the high proportion of patients with unclassified epilepsies in our series, the results support the use of a multigene epilepsy panel for a hypothesis-free diagnostic approach. Despite the fact that the clinical presentations of the epileptic disorders frequently overlap and even when phenotypic data are scarce, this type of approach, which includes the most relevant epilepsy-associated genes, offers rapid testing with a good diagnostic yield.

In conclusion, our HaloPlex design demonstrates the utility of this gene panel approach to identify the cause of cases with some type of genetic epilepsy in infancy. The early identification of the underlying causative genetic alteration using NGS approach will provide prognostic information, influence therapeutic decisions and lead to the design of new drugs targeted to gene-specific defects [9, 10, 73, 74].

Supporting information

S1 Table. Genes in the first panel of epilepsy.

(DOC)

S2 Table. Genes in the second panel of epilepsy.

(DOC)

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References

1. Tuchman R, Moshe SL, Rapin I. Convulsing toward the pathophysiology of autism. Brain & development. 2009; 31(2):95–103. Epub 2008/11/14.
2. Dulac O. Epileptic encephalopathy. Epilepsia. 2001; 42 Suppl 3:23–6. Epub 2001/08/25.
3. Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on
4. Holland KD, Hallinan BE. What causes epileptic encephalopathy in infancy?: the answer may lie in our genes. Neurology. 2010; 75(13):1132–3. Epub 2010/09/30. https://doi.org/10.1212/WNL.0b013e1811f6bc97 PMID: 20876466

5. Gursoy S, Ercal D. Diagnostic Approach to Genetic Causes of Early-Onset Epileptic Encephalopathy. Journal of child neurology. 2016; 31(4):523–32. Epub 2015/08/15. https://doi.org/10.1177/0883073815599262 PMID: 26271793

6. Thomas RH, Berkovic SF. The hidden genetics of epilepsy—a clinically important new paradigm. Nature reviews Neurology. 2014; 10(5):283–92. Epub 2014/04/16. https://doi.org/10.1038/nrneurol.2014.62 PMID: 24733163

7. Mastrangelo M, Leuzzi V. Genes of early-onset epileptic encephalopathies: from genotype to phenotype. Pediatr Neurol. 2012; 46(1):24–31. Epub 2011/12/27. https://doi.org/10.1016/j.pediatrneurol.2011.11.003 PMID: 22196487

8. Lemke JR, Riesch E, Scheurenbrand T, Schubach M, Wilhelm C, Steiner I, et al. Targeted next generation sequencing as a diagnostic tool in epileptic disorders. Epilepsia. 2012; 53(8):1387–98. Epub 2012/05/23. https://doi.org/10.1111/j.1528-1167.2012.03516.x PMID: 22612257

9. Orsini A, Zara F, Striano P. Recent advances in epilepsy genetics. Neuroscience letters. 2017. Epub 2017/05/14.

10. Weber YG, Biskup S, Helbig KL, Von Spiczak S, Lerche H. The role of genetic testing in epilepsy diagnosis and management. Expert review of molecular diagnostics. 2017; 17(8):739–50. Epub 2017/05/27. https://doi.org/10.1080/14737159.2017.1335598 PMID: 28548558

11. Wang J, Lin ZJ, Liu L, Xu HQ, Shi YW, Yi YH, et al. Epilepsy-associated genes. Seizure. 2012; 53(8):1387–98. Epub 2012/05/23. https://doi.org/10.1111/j.1528-1167.2012.03516.x PMID: 22612257

12. Hildebrand MS, Dahl HH, Damiano JA, Smith RJ, Scheffer IE, Berkovic SF. Recent advances in the molecular genetics of epilepsy. Journal of medical genetics. 2013; 50(5):271–9. Epub 2013/03/08. https://doi.org/10.1136/jmedgenet-2012-101448 PMID: 23468209

13. Hirose S, Scheffer IE, Marini C, De Jonghe P, Andermann E, Goldman AM, et al. SCN1A testing for epilepsy: application in clinical practice. Epilepsia. 2013; 54(5):946–52. Epub 2013/04/17. https://doi.org/10.1111/epi.12168 PMID: 23586701

14. Pong AW, Pal DK, Chung WK. Developments in molecular genetic diagnostics: an update for the pediatric epilepsy specialist. Pediatric neurology. 2011; 46(1):24–31. Epub 2011/12/27. https://doi.org/10.1016/j.pediatrneurol.2011.11.003 PMID: 22196487

15. Michaud JL, Lachance M, Hamdan FF, Carmant L, Noebels JL, et al. Genetic testing in the epilepsies—report of the ILAE Genetics Commission. Epilepsia. 2010; 51(4):523–32. Epub 2010/09/30. https://doi.org/10.1111/j.1528-1167.2010.02522.x PMID: 20196795

16. Veeramah KR, Johnstone L, Karafet TM, Wolf D, Sprissler R, Salogiannis J, et al. Exome sequencing reveals new causal mutations in children with epileptic encephalopathies. Epilepsia. 2013; 54(7):1270–81. Epub 2013/05/08. https://doi.org/10.1111/epi.12201 PMID: 23647072

17. Dimassi S, Labalme A, Ville D, Calender A, Mignot C, Boutry-Kryza N, et al. Whole-exome sequencing improves the diagnosis yield in sporadic infantile spasms syndrome. Clinical genetics. 2016; 89(2):198–204. Epub 2015/07/04. https://doi.org/10.1111/cge.12636 PMID: 26138355

18. Epi4K Consortium. Epi4K: gene discovery in 4,000 genomes. Epilepsia. 2012; 53(8):1457–67. Epub 2012/05/31. https://doi.org/10.1111/j.1528-1167.2012.03511.x PMID: 22642626

19. Poduri A, Sheidley BR, Shostak S, Ottman R. Genetic testing in the epilepsies—development and dilemmas. Nature reviews Neurology. 2014; 10(5):293–9. Epub 2014/04/16. https://doi.org/10.1038/nrneurol.2014.60 PMID: 24733164

20. Scheffer IE. Genetic testing in epilepsy: what should you be doing? Epilepsy currents / American Epilepsy Society. 2011; 11(4):107–11. Epub 2011/08/13.

21. Michaud JL, Lachance M, Hamdan FF, Carmant L, Lortie A, Diadori P, et al. The genetic landscape of infantile spasms. Human molecular genetics. 2014; 23(18):4846–58. Epub 2014/05/02. https://doi.org/10.1093/hmg/ddu199 PMID: 24781210

22. Martín HC, Kim GE, Pagnotamma AT, Murakami Y, Carvill GL, Meyer E, et al. Clinical whole-genome sequencing in severe early-onset epilepsy reveals new genes and improves molecular diagnosis. Human molecular genetics. 2014; 23(12):3200–11. Epub 2014/01/28. https://doi.org/10.1093/hmg/ddu030 PMID: 24436883

23. Della Mina E, Ciccone R, Brustia F, Bayindir B, Limongelli I, Vetro A, et al. Improving molecular diagnosis in epilepsy by a dedicated high-throughput sequencing platform. European journal of human
Molecular diagnosis by a customized panel of epilepsy genes

genetics: EJHG. 2015; 23(3):354–62. Epub 2014/05/23. https://doi.org/10.1038/ejhg.2014.92 PMID: 24848745

24. Kwong AK, Ho AC, Fung CW, Wong VC. Analysis of mutations in 7 genes associated with neuronal excitability and synaptic transmission in a cohort of children with non-syndromic infantile epileptic encephalopathy. PloS one. 2015; 10(5):e0126446. Epub 2015/05/08. https://doi.org/10.1371/journal.pone.0126446 PMID: 25951140

25. Coll M, Allegue C, Partenzi S, Mates J, Del Olmo B, Campuzano O, et al. Genetic investigation of sudden unexpected death in epilepsy cohort by panel target resequencing. International journal of legal medicine. 2016; 130(2):331–9. Epub 2015/10/02. https://doi.org/10.1007/s00414-015-1269-0 PMID: 26429392

26. Moller RS, Larsen LH, Johannesen KM, Talvik I, Talvik T, Vahe U, et al. Gene Panel Testing in Epileptic Encephalopathies and Familial Epilepsies. Molecular syndromology. 2016; 7(4):210–9. Epub 2016/10/27. https://doi.org/10.1159/000483609 PMID: 27781031

27. Parrini E, Marinì D, Galuppi A, Cellini E, Pucatti D, et al. Diagnostic Targeted Resequencing in 349 Patients with Drug-Resistant Pediatric Epilepsies Identifies Causative Mutations in 30 Different Human mutation. 2016. Epub 2016/11/20.

28. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009; 25(14):1754–60. Epub 2009/05/20. https://doi.org/10.1093/bioinformatics/btp324 PMID: 19451168

29. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome research. 2010; 20(9):1287–303. Epub 2010/07/21. https://doi.org/10.1101/gr.107524.110 PMID: 20644199

30. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nature genetics. 2011; 43(5):491–8. Epub 2011/04/12. https://doi.org/10.1038/ng.806 PMID: 21478889

31. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Current protocols in bioinformatics / editorial board, Andreas D Baxevanis [et al]. 2013; 43:11 0 1–33. Epub 2014/11/29.

32. Garrison E, Marth G. Haplotype-based variant detection from short-read sequencing. Preprint at arXiv:1207.3907v2 [q-bioGN]. 2012.

33. Desmet FO, Hamroun D, Lalande M, Collod-Beroud G, Claustres M, Beroud C. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. Nucleic acids research. 2009; 37(9):e67. Epub 2009/04/03. https://doi.org/10.1093/nar/gkp215 PMID: 19339519

34. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nature methods. 2010; 7(4):248–9. Epub 2010/04/01. https://doi.org/10.1038/nmeth0410-248 PMID: 20354512

35. Schwarz JM, Rodelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nature methods. 2010; 7(8):575–6. Epub 2010/08/03. https://doi.org/10.1038/nmeth0810-575 PMID: 20676075

36. Robinson PN, Kohler S, Oellrich A, Wang K, Mungall CJ, Lewis SE, et al. Improved exome prioritization of disease genes through cross-species phenotype comparison. Genome research. 2014; 24(2):340–8. Epub 2013/10/29. https://doi.org/10.1101/gr.160325.113 PMID: 24162188

37. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nature protocols. 2009; 4(7):1073–81. Epub 2009/11/29. https://doi.org/10.1038/nmeth0909-248 PMID: 19561590

38. Cooper GM, Stone EA, Asimenos G, Green ED, Batzoglou S, Sidow A. Distribution and intensity of constraint in mammalian genomic sequence. Genome research. 2005; 15(7):901–13. Epub 2005/06/21. https://doi.org/10.1101/gr.3577405 PMID: 15965027

39. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in medicine: official journal of the American College of Medical Genetics. 2015; 17(5):405–24. Epub 2015/03/06.

40. Weckhuysen S, Ivanovic V, Hendrickx R, Van Coster R, Hjalgrim H, Moller RS, et al. Extending the KCNQ2 encephalopathy spectrum: clinical and neuroimaging findings in 17 patients. Neurology. 2013; 81(19):1697–703. Epub 2013/10/11. https://doi.org/10.1212/01.wnl.0000436296.72400.a1 PMID: 24107868

41. Romaniello R, Saettini F, Panzeri A, Arrigoni F, Bassi MT, Borgatti R. A de-novo STXBP1 gene mutation in a patient showing the Rett syndrome phenotype. Neuroreport. 2015; 26(5):254–7. Epub 2015/02/26. https://doi.org/10.1097/WNR.0000000000000337 PMID: 25714420
Molecular diagnosis by a customized panel of epilepsy genes

42. Saitsu H, Kato M, Okada I, Orii KE, Higuchi T, Hoshino H, et al. STXBP1 mutations in early infantile epileptic encephalopathy with suppression-burst pattern. Epilepsia. 2010; 51(12):2397–405. Epub 2010/10/05. https://doi.org/10.1111/j.1528-1167.2010.02728.x PMID: 20887364

43. Bahi-Buisson N, Villeeneuve N, Caietta E, Jacquette A, Maurey H, Matthys G, et al. Recurrent mutations in the CDKL5 gene: genotype-phenotype relationships. American journal of medical genetics Part A. 2012; 158A(7):1612–9. Epub 2012/06/09. https://doi.org/10.1002/ajmg.a.35401 PMID: 22678952

44. Ataman ZA, Gakhtar L, Sorenson BR, Helbig KL, Farwell Hagman KD, Shinde DN, Mroske C, Powis Z, Li S, et al. Diagnostic exome sequencing provides a molecular diagnosis for a significant proportion of patients with epilepsy. Genet Med. 2014; 16(7):360–70. Epub 2014/09/30. https://doi.org/10.1016/j.gene.2014.08.013 PMID: 25262651

45. van Harsseleer VJ, Weckhuyzen S, van Kempen MJ, Hardies K, Verbeek NE, de Kovel CG, et al. Clinical and genetic aspects of CDH19-related epilepsy syndromes and the possible role of CDH19 mutations in males with autism spectrum disorders. Neurogenetics. 2013; 14(1):23–34. Epub 2013/01/22. https://doi.org/10.1007/s10048-013-0533-1 PMID: 23334464

46. Marin C, Darra F, Specchio N, Mei D, Terracciano A, Parmeggiani L, et al. Focal seizures with affective symptoms are a major feature of PCDH19 gene-related epilepsy. Epilepsia. 2012; 53(12):2119–9. Epub 2012/09/06. https://doi.org/10.1111/j.1528-1167.2012.03649.x PMID: 22946748

47. van Hassel JJ, Weckhuyzen S, van Kempen MJ, Hardies K, Verbeek NE, de Kovel CG, et al. Clinical and genetic aspects of CDH19-related epilepsy syndromes and the possible role of CDH19 mutations in males with autism spectrum disorders. Neurogenetics. 2013; 14(1):23–34. Epub 2013/01/22. https://doi.org/10.1007/s10048-013-0533-1 PMID: 23334464

48. Scheffer IE. Epilepsy genetics revolutionizes clinical practice. Neuropediatrics. 2014; 45(2):70–4. Epub 2014/03/13. https://doi.org/10.1055/s-0034-1371508 PMID: 24615646

49. Carvilli G, Heavin SB, Yendle SC, McMahon JM, O’Roak BJ, Cook J, et al. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in CHD2 and SYNGAP1. Nature genetics. 2013; 45(7):825–30. Epub 2013/05/28. https://doi.org/10.1038/ng.2646 PMID: 23708187

50. Hildebrand MS, Myers CT, Carvilli G, Regan BM, Damiano JA, Mullen SA, et al. A targeted resequencing gene panel for focal epilepsy. Neurology. 2016; 86(17):1605–12. Epub 2016/04/01. https://doi.org/10.1212/wnl.000000000002608 PMID: 27029629

51. Kodera H, Kato M, Nord AS, Walsh T, Lee M, Yamanaka G, et al. Targeted capture and sequencing for detection of mutations causing early onset epileptic encephalopathy. Epilepsia. 2013; 54(7):1262–9. Epub 2013/05/15. https://doi.org/10.1111/epi.12203 PMID: 23662938

52. Hata Y, Yoshiida K, Kinoshita K, Nishida N. Epilepsy-Related Sudden Unexpected Death: Targeted Molecular Analysis of Inherited Heart Disease Genes using Next-Generation DNA Sequencing. Brain Pathol. 2016. Epub 2016/05/03.

53. Hart D, Hwang SJ, McGeehan S, McManus A, Parry-Jones D, Myles JS, et al. Improving diagnosis and broadening the phenotypes in early-onset seizure and severe developmental delay disorders through gene panel analysis. Journal of medical genetics. 2016. Epub 2016/03/20.

54. Wang W, Wang C, Dawson DB, Thorland EC, Lundquist PA, Eckloff BW, et al. Target-enrichment sequencing and copy number evaluation in inherited polyneuropathy. Neurology. 2016; 86(19):1762–71. Epub 2016/05/11. https://doi.org/10.1212/wnl.000000000002689 PMID: 27164712

55. Mercimek-Mahmutoglu S, Patel J, Cordeiro D, Hewson S, Callen D, Donner EJ, et al. Diagnostic yield of genetic testing in epileptic encephalopathy in childhood. Epilepsia. 2015; 56(5):707–16. Epub 2015/05/11. https://doi.org/10.1111/epi.12203 PMID: 23662938

56. Allen AS, Berkovic SF, Cossette P, Delanty N, Dlugos D, Eichler EE, et al. No novo mutations in epileptic encephalopathies. Nature. 2013; 501(7466):217–21. Epub 2013/08/13. https://doi.org/10.1038/nature12439 PMID: 23934111

57. EuroEPINOMICS-RES Consortium EPgp, and Epi4K Consortium. De novo mutations in synaptic transmission genes including DNMF1 cause epileptic encephalopathies. American journal of human genetics. 2014; 95(4):360–70. Epub 2014/09/30. https://doi.org/10.1016/j.ajhg.2014.08.013 PMID: 25262651

58. Helbig KL, Fanwell Hagman KD, Shinde DN, Mroske C, Powis Z, Li S, et al. Diagnostic exome sequencing provides a molecular diagnosis for a significant proportion of patients with epilepsy. Genetics in medicine: official journal of the American College of Medical Genetics. 2016. Epub 2016/01/23.

59. Singh NA, Charlier C, Stauffer D, DuPont BR, Leach RJ, Melis R, et al. A novel potassium channel gene, KON2, is mutated in an inherited epilepsy of newborns. Nature genetics. 1998; 18(1):25–9. Epub 1998/01/13. https://doi.org/10.1038/ng1998-25 PMID: 9425895

60. Kalischuer VM, Tao J, Donnelly A, Hollway G, Schwinger E, Kubart S, et al. Disruption of the serine/threonine kinase 9 gene causes severe X-linked infantile spasms and mental retardation. American journal of human genetics. 2003; 72(6):1401–11. Epub 2003/05/08. https://doi.org/10.1086/375538 PMID: 12736870
61. Saitsu H, Kato M, Mizuguchi T, Hamada K, Osaka H, Tohyama J, et al. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. Nature genetics. 2008; 40(6):782–8. Epub 2008/05/13. https://doi.org/10.1038/ng.150 PMID: 18469812

62. Baulac S, Gourfinkel-An I, Picard F, Rosenberg-Bourgin M, Prud'homme JF, Baulac M, et al. A second locus for familial generalized epilepsy with febrile seizures plus maps to chromosome 2q21-q33. American journal of human genetics. 1999; 65(4):1078–85. Epub 1999/09/16. https://doi.org/10.1086/302593 PMID: 10486327

63. Escayg A, MacDonald BT, Meisler MH, Baulac S, Huberfeld G, An-Gourfinkel I, et al. Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS+2. Nature genetics. 2000; 24(4):343–5. Epub 2000/03/31. https://doi.org/10.1038/74159 PMID: 10742094

64. Dibbens LM, Tarpey PS, Hynes K, Bayly MA, Scheffer IE, Smith R, et al. X-linked protocadherin 19 mutations cause female-limited epilepsy and cognitive impairment. Nature genetics. 2008; 40(6):776–81. Epub 2008/05/13. https://doi.org/10.1038/ng.149 PMID: 18469813

65. Van Goethem G, Mercelis R, Lofgren A, Seneca S, Ceuterick C, Martin JJ, et al. Patient homozygous for a recessive POLG mutation presents with features of MERRF. Neurology. 2003; 61(12):1811–3. Epub 2003/12/25. PMID: 14694057

66. Cornford EM, Hyman S, Cornford ME, Landaw EM, Delgado-Escueta AV. Interictal seizure resections show two configurations of endothelial Glut1 glucose transporter in the human blood-brain barrier. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism. 1998; 18(1):26–42. Epub 1998/01/15.

67. Stromme P, Mangelsdorf ME, Shaw MA, Lower KM, Lewis SM, Bruyere H, et al. Mutations in the human ortholog of Aristless cause X-linked mental retardation and epilepsy. Nature genetics. 2002; 30(4):441–5. Epub 2002/03/13. https://doi.org/10.1038/ng862 PMID: 11889467

68. Capelli LP, Krepischi AC, Gurgel-Giannetti J, Mendes MF, Rodrigues T, Varela MC, et al. Deletion of the RMGA and CHD2 genes in a child with epilepsy and mental deficiency. European journal of medical genetics. 2012; 55(2):132–4. Epub 2011/12/20. https://doi.org/10.1016/j.ejmg.2011.10.004 PMID: 22178256

69. Zollino M, Gumieri F, Orteschi D, Marangi G, Leuzzi V, Neri G. Integrated analysis of clinical signs and literature data for the diagnosis and therapy of a previously undescribed 6p21.3 deletion syndrome. European journal of human genetics: EJHG. 2011; 19(2):239–42. Epub 2010/12/02. https://doi.org/10.1038/ejhg.2010.172 PMID: 21119708

70. Ding YX, Zhang Y, He B, Yue WH, Zhang D, Zou LP. A possible association of responsiveness to adrenocorticotropic hormone with specific GRIN1 haplotypes in infantile spasms. Developmental medicine and child neurology. 2010; 52(11):1028–32. Epub 2010/08/21. https://doi.org/10.1111/j.1469-8749.2010.03746.x PMID: 20722663

71. Allen NM, Conroy J, Shahwan A, Lynch B, Correa RG, Pena SD, et al. Unexplained early onset epileptic encephalopathy: Exome screening and phenotype expansion. Epilepsia. 2016; 57(1):e12–7. Epub 2015/12/10. https://doi.org/10.1111/epi.13250 PMID: 26648591

72. Kobayashi Y, Tohyama J, Kato M, Akasaka N, Magara S, Kawashima H, et al. High prevalence of genetic alterations in early-onset epileptic encephalopathies associated with infantile movement disorders. Brain & development. 2016; 38(3):285–92. Epub 2015/10/21.

73. Striano P, Vari MS, Mazzocchetti C, Verrotti A, Zara F. Management of genetic epilepsies: From empirical treatment to precision medicine. Pharmacological research. 2016; 107:426–9. Epub 2016/04/16. https://doi.org/10.1016/j.phrs.2016.04.006 PMID: 27080588

74. Symonds JD, Zuberi SM, Johnson MR. Advances in epilepsy gene discovery and implications for epilepsy diagnosis and treatment. Current opinion in neurology. 2017; 30(2):193–9. Epub 2017/02/18. https://doi.org/10.1097/WCO.0000000000000433 PMID: 28212175