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Gene Panel Testing in Epileptic Encephalopathies and Familial Epilepsies

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Key Words

Epileptic encephalopathies · Familial epilepsies · Gene panel testing · Seizures

Abstract

In recent years, several genes have been causally associated with epilepsy. However, making a genetic diagnosis in a patient can still be difficult, since extensive phenotypic and genetic heterogeneity has been observed in many monogenic epilepsies. This study aimed to analyze the genetic basis of a wide spectrum of epilepsies with age of onset spanning from the neonatal period to adulthood. A gene panel targeting 46 epilepsy genes was used on a cohort of 216 patients consecutively referred for panel testing. The patients had a range of different epilepsies from benign neonatal seizures to epileptic encephalopathies (EEs). Potentially causative variants were evaluated by literature and database searches, submitted to bioinformatic prediction algorithms, and validated by Sanger sequencing. If possible, parents were included for segregation analysis. We identified a presumed disease-causing variant in 49 (23%) of the 216 patients. The

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variants were found in 19 different genes including *SCN1A, STXBP1, CDKL5, SCN2A, SCN8A, GABRA1, KCNA2,* and *STX1B*. Patients with neonatal-onset epilepsies had the highest rate of positive findings (57%). The overall yield for patients with EEs was 32%, compared to 17% among patients with generalized epilepsies and 16% in patients with focal or multifocal epilepsies. By the use of a gene panel consisting of 46 epilepsy genes, we were able to find a disease-causing genetic variation in 23% of the analyzed patients. The highest yield was found among patients with neonatal-onset epilepsies and EEs.

During the last decade, great progress has been made in epilepsy genetics. This has primarily been technology driven due to the development of massive parallel sequencing. The extreme drop in sequencing costs has made it feasible to do whole-exome sequencing (WES) on family trios, which has led to the discovery of several novel epilepsy-related genes and elucidated new molecular pathways [for review, see McTague et al., 2016]. At the same time, it has been proposed by the International League Against Epilepsy Commission on terminology to replace the term 'idiopathic' with the term 'genetic', acknowledging the role of genetic factors in the large group of previously labeled idiopathic epilepsies [Berg et al., 2010].

The group of genetic epilepsies has a wide phenotypic spectrum, ranging from mild treatable seizure disorders in individuals with normal cognition to severe early-onset epileptic encephalopathies (EEs) associated with profound intellectual disability (ID). The genetic etiology of the different epilepsies is just as heterogeneous as the phenotypic spectrum, ranging from monogenic causes with little or no influence from modifiers or environmental factors to genetically complex forms involving multiple genes, modifiers and likely environmental factors [Thomas and Berkovic, 2014]. Extensive phenotypic and genetic heterogeneity has been observed in many monogenic epilepsies, meaning that genotype-phenotype correlations are not always straightforward. The same gene and even the same mutation can lead to broad phenotypic variations, and the same epilepsy syndrome can be caused by mutations in different genes [Depienne et al., 2011; Marini et al., 2011; Carvill et al., 2014; Goldberg-Stern et al., 2014; Møller et al., 2015]. This illustrates the insufficiency of the previous single gene test approach and the need for a multigene next-generation sequencing (NGS)-based strategy, either as full-genome sequencing, WES, or targeted gene panels.

Here, we report the results of screening by a gene panel containing 46 known epilepsy genes on a cohort of 216 patients with various forms of epilepsy, and we discuss the yields of such an approach from a diagnostic and therapeutic perspective.

Patients and Methods

Patients

The patients included in the study were probands referred with various forms of epilepsy from Denmark, Estonia, the UK, Argentina, and Pakistan, which were sent for genetic analysis over a period of time of 16 months from March 2014 to July 2015. The cohort consisted of 216 patients with a diverse range of EE phenotypes or milder familial epilepsies. The age at referral was between 2 weeks and 74 years of age. Forty-nine (23%) of the patients were over 18 years of age. Seizure and medical histories provided by the referring physicians were evaluated, and, if possible, epilepsy syndromes were classified according to the International League Against Epilepsy [Berg et al., 2010]. The majority of the patients (n = 112; 52%) had different EEs, including Ohtahara syndrome, West syndrome, malignant migrating partial seizures of infancy, and unspecified EEs with seizure onset within the first years of life. Forty-four (20%) of the patients had multifocal or focal epilepsies including benign familial neonatal or infantile seizures (BFIS) and autosomal dominant nocturnal frontal lobe epilepsy. Twenty-three (11%) patients had generalized epilepsies, including genetic generalized epilepsy, generalized epilepsy with febrile seizures+ (GEFS+), myoclonic atonic epilepsy, or early-onset absence epilepsy. The remaining patients had unclassified epilepsies with or without ID. One patient had only recurrent long-lasting febrile seizures (FS+) and one patient turned out to have benign myoclonus of infancy instead of epilepsy. Detailed clinical information was not available for 19 patients. Less than 10% of the patients had previously undergone negative mutation screening for selected epilepsy genes, e.g., *SCN1A* and *STXBP1*.

The Gene Panel

To identify presumed disease-causing variants, we performed targeted NGS of 46 epilepsy genes. The criteria for including a gene on the panel were that it should have been reported more than once in patients with monogenic epilepsies. The selection of genes was made in January 2014, meaning that some genes discovered in the past 2 years were not included on this panel – exceptions are some unpublished gene discoveries the authors were aware of when the panel was developed, e.g., *KCNA2*, *STX1B*, *GABRA1*, *DNM1*, and *HCN1* [Carvill et al., 2014; EuroEPINOMICS-RES Consortium et al., 2014; Nava et al., 2014; Schubert et al., 2014; Syrbe et al., 2015]. The genes included on the present panel are: *ALDH7A1*, *ALG13*, *ARHGEF9*, *CACNA1A*, *CDKL5*, *CHD2*, *CPA6*, *DEPDC5*, *DNM1*, *GABRA1*, *GABBR1*, *GABBR2*, *GABRB3*, *GABRD*, *GABRG2*, *GNAO1*, *GRIN1*, *GRIN2A*, *GRIN2B*, *HCN1*, *HDAC4*, *HNRNPU*, *IQSEQ2*, *KCNA2*, *KCNQ2*, *KCNQ3*, *KCNT1*, *KCTD7*, *LGI1*, *MBD5*, *PDCH19*, *PLCB1*, *PNPO*, *PRRT2*, *SCN1A*, *SCN1B*, *SCN2A*, *SCN8A*, *SLC25A22*, *SLC2A1*, *SLC35A3*, *SPTAN1*, *STX1B*, *STXBP1*, *SYNGAP1*, and *TBC1D24*.

Sample Preparation

Genomic DNA was extracted from full blood using standard methods. Targeted NGS libraries were prepared from 15 ng of template DNA using the Ion AmpliSeq library 2.0 kit and custom primers following the manufacturer's instructions (Life Technologies). Sequencing adaptors with index sequences (barcodes) that enable sample multiplexing were ligated to the amplicons using the Ion Xpress barcode adaptor kit (Life Technologies). The library DNA was clonally amplified onto the Ion Spheres Particles (ISPs) by emulsion PCR using an Ion OneTouch 2 system and the Ion PGM Template OT2 200 kit (Life Technologies). ISPs were sequenced on an Ion PGM sequencer using an Ion 314 or Ion 316 chip and the Ion PGM 200 Sequencing kit as per the manufacturer's instructions (Life Technologies).

Bioinformatics

Sequences were mapped to hg19 in the Torrent suite software (Life Technologies) and variant calling was achieved in the Strand NGS software (Avadis) with a minimum of 20-fold read depth. Common SNPs with an allele frequency ≥2% and SNPs observed in more than 2 samples for each analyzed sample batch were filtered out. Genetic nonsynonymous/splice site variants were evaluated through database searches such as dbSNP, Exome Variant Server, the Exome Aggregation Consortium database (ExAC), and HGMD Professional. Missense variants were also submitted to prediction softwares such as SIFT and PolyPhen-2, while splice site variants were evaluated by NNSPlice and Splicesite finder. Variants analyzed under a dominant inheritance model that were observed more than 10 times in ExAC were considered too common and discarded. Potentially pathogenic variants were validated through conventional Sanger sequencing, and, if possible, parents were included for segregation analysis.

Criteria for Pathogenicity of Rare Variants

For possibly damaging variants where segregation analysis could be performed, we required the variant to meet one of the following criteria to constitute a possible pathogenic variant: it arose de novo, segregated with the disorder, was inherited from a parent with somatic mosaicism, was inherited from an unaffected parent but previously reported in other families with the same phenotype and incomplete penetrance, or adhered to a recessive X-linked or parent-of-origin mode of inheritance. Paternity testing was not performed.

Results

The overall target coverage of the 46 genes was 95–97%; hence, 3–5% of the regions were not analyzed, and some variations may be missed for this reason. The regions missed were more or less identical across the different samples, i.e., regions difficult to amplify due to high GC content, repeat elements, or regions with homology in other parts of the genome. We identified presumed disease-causing variations in 49 of 216 patients (table 1), corresponding to a diagnostic yield of 23%. The variations constituted both known and novel variants in 19 different genes, and all were predicted damaging by one or more softwares.

Twenty-one patients had neonatal-onset epilepsies, e.g., benign familial neonatal seizures or neonatal-onset EEs, and presumed pathogenic variants were found in 12 of these cases corresponding to a yield of 57%. In comparison, only 4/29 (14%) patients with seizure onset between 2 and 9 years of age had a positive finding, and no disease-causing variants were found among 9 patients with seizure onset between 10 and 28 years. The epilepsy phenotypes in the 2 latter groups included myoclonic atonic epilepsy, genetic generalized epilepsy (childhood absence epilepsy, juvenile absence epilepsy, and juvenile myoclonic epilepsy), and focal or multifocal epilepsies. The remaining 138 patients for whom data on seizure onset was available had onset between 2 months and 2 years and a presumed pathogenic variant was found in 36 (26%) of them.

Thirty-six of the 49 presumed pathogenic variants were found among 112 patients with EEs (yield: 32%). In comparison, 7 variants were detected in 44 patients with focal or multifocal epilepsies including benign familial neonatal seizures, BFIS, and autosomal dominant nocturnal frontal lobe epilepsy (yield: 16%), and 4 variants were detected among 23 patients with generalized epilepsies, e.g., GEFS+ (yield: 17%). The remaining 2 presumed disease-causing variants were found in patients with FS+ or unclassified epilepsy and ID, respectively. Six variants had been seen between 1 and 5 times in ExAC. Four of these were found in patients with relatively mild phenotypes: FS+ *(SCN1A)*, GEFS+ *(SCN1A)* or focal epilepsy *(CPA6* and *KCNT1)*, while another was one of a pair of compound heterozygous variants in a recessive gene *(PNPO)*.

Segregation analysis was performed in 33 of the 49 cases; 29 of the variants occurred de novo, 2 were homozygous or compound heterozygous *(PNPO*, and *PRRT2)*, 3 were inherited from an affected parent (*KCNT1*, *GABRA1*, and *KCNQ2*), and 1 variant *(SLC2A1)* was inherited from an unaffected parent. This particular *SLC2A1* variant has previously been reported in a family with paroxysmal exertion-induced dyskinesias, epilepsy, mild ID, impulsivity, and incomplete penetrance [Weber et al., 2008]. Parental DNA was not available for the remaining 16 presumed disease-causing variants. However, the majority of these 16 variants were either frameshift, nonsense, or splice site variants observed in known EE genes, e.g., *SCN1A*, *STXBP1*, and *CDKL5* (7/16 variants) or missense variants that have previously been reported in patients affected by the same epilepsy syndrome as observed in the proband (5/16 variants).

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Table 1. Presumed disease-causing mutations

Sample ID	Phenotype	Gene	cDNA	Protein position	Inheritance	Variation previously published according to HGMD	SIFT	PolyPhen	ExAC	Patient previously published
EG1371	MMPSI	*CACNA1A*	c.301G>C	p.Glu101Gln	de novo	no	not tolerated	probably damaging	no	Epi4K Consortium, 2016
EG1711	EOEE	*CDKL5*	c.577G>C	p.Asp193His	de novo	no, but c.578A>G, p.Asp193Gly: Mirzaa et al., 2013	not tolerated	probably damaging	no	no
EG1979	EOEE	*CDKL5*	c.1243dupA	p.Thr415Asnfs*4	de novo	no			no	no
E00642279	EOEE	*CDKL5*	c.2152+1G>A		NA	no			no	no
EG1740	EOEE	*CDKL5*	c.2152+1G>T		de novo	no			no	no
EG1996	EE	*CHD2*	c.1880_1883delCTTT	p.Ser627Tyrfs*6	NA	no			no	no
E00729876	focal epilepsy	*CPA6*	c.1199G>A	p.Arg400His	de novo	no	not tolerated	probably damaging	2/121390	no
EG2111	GEFS+	*GABRA1*	c.220G>A	p.Val74Ile	paternal (affected)	no	not tolerated	probably damaging	no	no
EG1880	GEFS+/ Dravet	*GABRA1*	c.752G>A	p.Gly251Asp	de novo	no, but c.751G>A, p.Gly251Ser: Carvill et al., 2014	not tolerated	probably damaging	no	no
EG1542	EOEE	*GABRB3*	c.767T>A	p.Leu256Gln	de novo	no	not tolerated	possibly damaging	no	Epi4K Consortium, 2016
E00338050	EOEE	*GABRB3*	c.905A>G	p.Tyr302Cys	de novo	Epi4K et al., 2013	not tolerated	probably damaging	no	no
E00644458	Ohtahara syndrome	*GNAO1*	c.692A>G	p.Tyr231Cys	de novo	no	not tolerated	probably damaging	no	Talvik et al., 2015
2640	ID, focal epilepsy	*GRIN2A*	c.1841A>G	p.Asn614Ser	de novo	no	tolerated	probably damaging	no	no
EG1987	EE	*KCNA2*	c.890G>A	p.Arg297Gln	mother negative	no	not tolerated	probably damaging	no	no
2432	EOEE	*KCNQ2*	c.766G>A	p.Gly256Arg	de novo	no	not tolerated	benign	no	no
EG2310	EOEE	*KCNQ2*	c.913_915delTTC	p.Phe395del	NA	Ishii et al., 2009			no	no
EG1771	EE	*KCNQ2*	c.1521dupA	p.Glu508Argfs*13	maternal (affected)	no			no	no
EG1607	ADNFLE	*KCNT1*	c.2782C>T	p.Arg928Cys	maternal (affected)	Heron et al., 2012; Kim et al., 2014a; Milligan et al., 2014; Møller et al., 2015	not tolerated	probably damaging	2/121110	Møller et al., 2015
EG1381	West syndrome	*PNPO*	c.98A>T c.533T>G	p.Asp33Val, p.Ile178Ser	c.98A>T maternal c.533T>G paternal	c.98A>T: Schmitt et al., 2010; Goyal et al., 2013; Mills et al., 2014; Sudarsanam et al., 2014	not tolerated	probably damaging	c.98A>T: 5/21304, c.533T>G: no	no
EG2202	BFIS, movement disorder	*PRRT2*	c.836C>T (homozygous)	p.Pro279Leu	NA	no	not tolerated	probably damaging	no	no
EG1849	Dravet	*SCN1A*	c.530G>A	p.Gly177Glu	NA	Nabbout et al., 2003			no	no
EG1677	Dravet	*SCN1A*	c.625dupC	p.Leu209Profs*68	NA	no			no	no
EG1970	GEFS+/ Dravet	*SCN1A*	c.1376A>G	p.Gln459Arg	de novo	no	tolerated	possibly damaging	no	no
EG2085	Dravet	*SCN1A*	c.2636T>C	p.Leu879Pro	NA	no	not tolerated	probably damaging	no	no
EG1954	Dravet	*SCN1A*	c.2824C>G	p.Leu942Val	NA	no, but c.2825T>C, p.Leu942Pro: Mancardi et al., 2006	not tolerated	probably damaging	no	no
2287	GEFS+	*SCN1A*	c.2837G>A	p.Arg946His	NA	Fukuma et al., 2004; Verbeek et al., 2011; Volkers et al., 2011; Wang et al., 2014	not tolerated	probably damaging	no	no
EG1976	Dravet	*SCN1A*	c.3094G>T	p.Glu1032*	NA	Rocca et al., 2010; Xiong et al., 2015			no	no
EG2044	Dravet	*SCN1A*	c.3439_3442del GAAAinsTGCTT	p.Glu1147Cysfs*4	NA	no			no	no

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Table 1 (continued)

Sample ID	Phenotype	Gene	cDNA	Protein position	Inheritance	Variation previously published according to HGMD	SIFT	PolyPhen	ExAC	Patient previously published
EG1738	GEFS+	*SCN1A*	c.4786C>T	p.Arg1596Cys	de novo	Harkin et al., 2007; Cherepanova et al., 2013; Hoffman-Zacharska et al., 2015; Kim et al., 2014b	not tolerated	probably damaging	1/116992	no
EG2001	Dravet	*SCN1A*	c.5260G>A	p.Gly1754Arg	father negative	Petrelli et al., 2012	not tolerated	probably damaging	no	no
EG0863	Dravet	*SCN1A*	c.5269G>A	p.Gly1757Arg	de novo	no	not tolerated	probably damaging	no	no
EG2212	FS+	*SCN1A*	c.5773G>A	p.Ala1925Thr	NA	no	tolerated	probably damaging	ExAC 4/121222	no
2656	MMPSI	*SCN2A*	c.640T>C	p.Ser214Pro	de novo	no	not tolerated	probably damaging	no	no
EG1974	BFNS	*SCN2A*	c.781G>A	p.Val261Met	de novo	Liao et al., 2010b	not tolerated	probably damaging	no	no
EG2099	BFNS	*SCN2A*	c.788C>T	p.Val263Met	de novo	Liao et al., 2010a; Touma et al., 2013	not tolerated	probably damaging	no	no
2092	Ohtahara syndrome	*SCN2A*	c.2567G>A	p.Arg856Gln	de novo	no, but c.2567G>T, p. Arg856Leu: Howell et al., 2015	not tolerated	probably damaging	no	no
EG1389	West syndrome	*SCN2A*	c.3955C>T	p.Arg1319Trp	de novo	no, but c.3956, p.Arg1319His: Berkovic et al., 2004; Misra et al., 2008; Zara et al., 2013	not tolerated	probably damaging	1/121210	no
E00338446	EE, autism	*SCN2A*	c.4581dupT	p.Val1528Cysfs*7	de novo	no			no	no
E00859940	EOEE	*SCN8A*	c.715A>T	p.Thr239Ser	de novo	no	tolerated	probably damaging	no	no
EG2076	EOEE	*SCN8A*	c.4594A>T	p.Ile1532Phe	de novo	no	not tolerated	probably damaging	no	no
EG2153	focal epilepsy, mild ID	*SCN8A*	c.5458C>T	p.Arg1820*	de novo	no			no	no
EG2126	EOEE	*SLC2A1*	c.143G>A	p.Trp48*	NA	no, but c.144G>A, p.Trp48*: Zorzi et al., 2008; Xiong et al., 2015			no	no
E00543752	EE, dystonia	*SLC2A1*	c.470delG	p.Gly157Alafs*34	NA	no			no	no
2662	epilepsy, ID	*SLC2A1*	c.940G>A	p.Gly314Ser	maternal (unaffected)	Weber et al., 2008; Deng et al., 2014	not tolerated	probably damaging	no	no
EG2060	GEFS+	*STX1B*	c.23_26dupTGCG	p.Ser10Alafs*7	de novo	no			no	no
EG1846	EE	*STXBP1*	c.794 + 5G>A		de novo	no			no	Weckhuysen et al., 2013
EG1940	EE	*STXBP1*	c.795-2A>T		de novo	no			no	Stamberger et al., 2016
2016	EE	*STXBP1*	c.1548-6_1559delinsAT		de novo	no			no	Stamberger et al., 2016
EG1780	EE	*STXBP1*	c.1723C>T	p.Pro575Ser	de novo	no	not tolerated	probably damaging	no	Stamberger et al., 2016

ADNFLE = Autosomal dominant nocturnal frontal lobe epilepsy; BFNS = benign familial neonatal seizures; EOEE = early-onset epileptic encephalopathy; MMPSI = malignant migrating partial epilepsy of infancy; NA = not analyzed.

Table 2. Variants of unknown significance

Sample ID	Phenotype	Gene	cDNA	Protein position	Inheritance	SIFT	PolyPhen	ExAC
E00858255	EE	*ALG13*	c.1641A>T	p.Gln547His	maternal	not tolerated	probably damaging	no
EG2199	West syndrome	*DNM1*	c.1344G>C	Gln448His	mother negative	not tolerated	benign	no
EP15-2	epilepsy, ID	*DNM1*	c.2078delC	p.Lys694Argfs*21	NA			no
2660	EE	*HNRNPU*	c.2197_2199delGGA	p.Arg733del	NA			no
EG2104	LGS	*KCNA2*	c.1070G>A	Gln357Arg	NA	tolerated	possibly damaging	no
2659	EOEE	*SCN2A*	c.1312G>A	p.Glu428Lys	NA	not tolerated	probably damaging	no
EG1932	GEFS+	*STX1B*	c.546G>A	p.Met182Ile	paternal	tolerated	benign	no

EOEE = Early-onset epileptic encephalopathy; LGS = Lennox-Gastaut syndrome; NA = not analyzed.

We identified multiple patients with disease-causing variants in the most common epilepsy genes *SCN1A* (n = 12), *SCN2A* (n = 6), *STXBP1* (n = 4), *CDKL5* (n = 4), *SLC2A1* (n = 3), *SCN8A* (n = 3), and *KCNQ2* (n = 3) accounting for 71% (35/49) of all mutation-positive individuals in our cohort. Furthermore, we found pathogenic variants in the relatively undescribed epilepsy genes *GABRA1* and *GABRB3* in 2 individuals each. 8 of the 49 presumed disease-causing variants have already been published in other studies [Møller et al., 2015; Talvik et al., 2015; Epi4K Consortium, 2016; Stamberger et al., 2016].

Three of the 12 *SCN1A* variants were found in patients with FS+ or GEFS+ syndrome, whereas the additional 9 *SCN1A* variants were associated with Dravet syndrome. Seven out of the 9 Dravet-associated alterations were found in adults between 20 and 53 years of age previously diagnosed with an unspecified EE and ID. All 7 individuals were retrospectively diagnosed with Dravet syndrome. Five of them were being treated with lamotrigine and/or carbamazepine, which were tapered off after the *SCN1A* variant was found. The changes in medication led to improved alertness and gait in 2 patients (p.Gly1757Arg, p.Glu1032*) and worsening of the seizure frequency or behavior in 2 patients (p.Gly177Glu, p.Leu942Val), whereas the condition was unchanged in the last patient.

Three de novo variants in *SCN8A* were identified in the present cohort, 2 missense variants (p.Thr239Ser, p.Ile1532Phe), and 1 nonsense mutation (p.Arg1820*). The 2 missense variants were found in 2 girls with severe early-onset EE, hypotonia, dyskinetic movements, and profound ID without any spoken language, a phenotype identical to previously published patients with *SCN8A* encephalopathy [Larsen et al., 2015]. Both girls were resistant to a variety of antiepileptic drugs, including valproate and levetiracetam. However, one of them (p.Ile1532Phe) was treated with oxcarbazepine, a sodium channel blocker (SCB), which gave a significant seizure reduction. Interestingly, we found, to the best of our knowledge, the first *SCN8A* nonsense mutation in a 5-year-old boy with mild ID and onset of mild myoclonic seizures at the age of 3 months. He initially had a few generalized tonic-clonic seizures (GTCS), but at present, only mild myoclonic seizures in the fingers and hands remain. The boy's EEG has shown epileptiform changes compatible with multifocal epilepsy. He has been treated with lamotrigine with moderate response.

Only 2 homozygous or compound heterozygous variants *(PNPO*, *PRRT2)* were identified. A homozygous variant in *PRRT2* (p.Pro279Leu) was found in a 16-month-old boy born to healthy unrelated Danish parents. There was no history of epilepsy in the family. He had onset of clusters of focal and generalized seizures at 3 months of age. His epilepsy was classified as benign infantile epilepsy, and seizure control was achieved with levetiracetam and valproate. Initial EEG recordings showed a focus of sharp waves in the right centro-parietal region, which normalized at 6 months. At the age of 6 months, he developed a movement disorder with head titubations, which improved after change from valproate to oxcarbazepine. He is currently developing but has mild delay in motor milestones.

In addition, to the 49 above-mentioned variants, we identified 7 (3%) variants of unknown significance (VUS; table 2). Segregation analysis was only performed for 2 of these variants *(STX1B* and *ALG13)*. The *STX1B* alteration was found in a girl with prolonged febrile seizures as well

as afebrile GTCS and inherited from her unaffected father and grandfather. Mutations in *STX1B* encoding syntaxin-1B have recently been found in sporadic cases and families with fever-associated epilepsy syndromes [Schubert et al., 2014]. Incomplete penetrance has been reported [Schubert et al., 2014], which makes the finding in this family difficult to interpret. The *ALG13* variant was observed in a male patient with Lennox-Gastaut syndrome and shown to be inherited from the unaffected mother and grandmother. It was predicted damaging by different softwares and not present in ExAC. However, unfortunately no additional male family members were available for testing, and we were therefore unable to classify pathogenicity of this variant.

Discussion

In the present study, we screened a cohort of 216 patients with various forms of epilepsies of suspected genetic cause. A presumed disease-causing variant was found in 23%, and a VUS was found in additional 3%. Targeted epilepsy gene panels are increasingly being used in research, and diagnostic laboratories and several studies using gene panels consisting of 35–265 genes have been published with diagnostic yields ranging between 10 and 48.5% [Lemke et al., 2012; Carvill et al., 2013a; Kodera et al., 2013; Wang et al., 2014; Della Mina et al., 2015; Mercimek-Mahmutoglu et al., 2015]. In these studies, there was a clear tendency towards higher positive rates in patients with early-onset epilepsies as well as in cases with severe phenotypes, and as expected, the observed de novo rate was high [Epi4K Consortium et al., 2013]. We identified a pathogenic variant in 57% of the patients with neonatal-onset epilepsies compared to only 14% of patients with onset between 2 and 9 years of age. No disease-causing variants were found among 9 patients with genetic generalized epilepsy, focal or unclassified epilepsy and onset between age 10 and 28 years. The overall diagnostic yield for patients with EEs was 32%, compared to 16% in patients with focal or multifocal epilepsy. These diagnostic yields are similar to published studies on other targeted NGS panels [Lemke et al., 2012; Carvill et al., 2013a; Kodera et al., 2013; Wang et al., 2014; Della Mina et al., 2015; Mercimek-Mahmutoglu et al., 2015].

Presumed disease-causing *SCN1A* variants were found in 7 adults who were retrospectively diagnosed with Dravet syndrome. Dravet syndrome is caused by *SCN1A* mutations that are usually believed to result in loss of function, leading to lack of sodium channel function in inhibitory interneurons. SCBs are therefore usually contraindicated in *SCN1A*-related Dravet syndrome. Five of the 7 identified adults were tapered off SCB based on their genetic profile. Two of them clearly benefited from this change in treatment with improvement in alertness and gait. However, surprisingly 2 of them had worsening of seizure frequency or behavior after tapering off lamotrigine. One patient experienced less GTCS but more focal seizures and changes in behavior (irritability), whereas the other patient had an increased frequency of GTCS. The 2 patients did not have further changes in their medications; thus, no additional drugs were added. Both patients had an *SCN1A* missense variant p.Gly177Glu and p.Leu942Val, which raises the question whether Dravet syndrome also can be caused by gain-of-function mutations as seen in EEs caused by other sodium channel genes, e.g., *SCN2A* and *SCN8A* [Kearney et al., 2001; Veeramah et al., 2012]. In the 2 patients harboring an *SCN8A* missense variant, we found, as previously reported [Wagnon and Meisler, 2015; Boerma et al., 2016; Møller and Johannesen, 2016], a good response to the SCB oxcarbazepine and a poor response to levetiracetam [Wagnon and Meisler, 2015]. The patient with the *SCN8A* nonsense mutation has been treated with lamotrigine, despite the fact that this variant is likely to cause a LOF loss of function of the channel. These findings indicate that a gene panel approach can provide data that in some cases can translate positively into clinical practice, whereas in other instances, they demand further preclinical investigations.

We also found a de novo variant in *GRIN2A* (p.Asn614Ser), encoding the NMDA receptor subunit GluN2A in a 2-year-old boy with severe ID, severe hypotonia, ataxia, and onset of intractable focal epilepsy at the age of 3 months. Mutations in *GRIN2A* have been found in a significant subset of patients with epilepsy-aphasia syndromes, comprising a phenotypic spectrum from atypical benign partial epilepsy to EEs with continuous spikes and waves during slow-wave sleep and Landau-Kleffner syndrome [Carvill et al., 2013b; Lemke et al., 2013; Lesca et al., 2013]. Furthermore, a few cases with severe early-onset EEs and severe ID as seen in the present case have been reported [Pierson et al., 2014]. The identified variant p.Asn614Ser is positioned directly at the tip of the re-entrant pore loop of GluN2A. Variants of this domain have been shown to result in a significant loss of the Mg^{2+} block resulting in a marked gain of channel function and severe neurodevelopmental delay [Endele et al., 2010]. Pierson et al. [2014] recently reported that treatment with an NMDA receptor blocker, memantine,

in a patient with intractable epilepsy and ID due to a *GRIN2A* gain-of-function mutation resulted in marked reduction of seizure frequency. However, the efficacy of memantine in patients with *GRIN2A* gain-of-function mutations remains to be confirmed in clinical trials.

Only 2 recessive variants were found in the present cohort (table 1). One of them was a homozygous *PRRT2* variant found in a 16-month-old boy with BFIS, movement disorder, and mild gross motor delay. Heterozygous *PRRT2* mutations have been associated with a spectrum of conditions including BFIS, infantile convulsions and choreoathetosis and paroxysmal kinesigenic dyskinesia. ID has been reported in less than 1% of patients with heterozygous *PRRT2* mutations, whereas >60% of the patients with homozygous or compound heterozygous *PRTT2* mutations present with ID or learning difficulties [for review, see Ebrahimi-Fakhari et al., 2015]. The present patient is developing but has mild gross motor delay, and due to his young age, it is not possible to evaluate his cognitive outcome.

A selected gene panel approach can be a comparable alternative to WES analysis. The benefit of a gene panel compared to WES is the targeted approach, meaning that only phenotypically relevant genes are included, which reduces or even eliminates incidental findings. In this study, we also observed a relatively low fraction of VUS (3%) compared to some recent WES studies where the observed VUS fraction was ~10% [Yang et al., 2014; Helbig et al., 2016]. Furthermore, the limited number of genes allows higher sequencing read depth at lower costs than WES and therefore better analysis quality. The drawback is that the panels have a predefined number of genes, and any later novel gene discoveries demand a whole new setup from data generation to analysis. The present panel, as most of the previously published panels, has a slightly lower diagnostic yield compared to a WES diagnostic, in which a genetic diagnosis can be found in 33–58% depending on the epilepsy phenotype [Helbig et al., 2016]. Here, we show 23% overall positive findings and 57% for patients with neonatal-onset epilepsies, indicating that for this particular subgroup not much is gained by an exome-wide approach. Compared to a smaller panel, a large panel including hundreds of genes is not always more informative and often adds more confusion with its higher VUS rate and inclusion of genes which are not or only marginally associated with the phenotype. Furthermore, there is also an ethical and consent issue in testing well-defined patients with a large panel containing genes not relevant for the phenotype under study because of the potential need to counsel for incidental findings. The challenging task for the molecular geneticist is therefore to design a panel with relevant genes. However, both the designer of the panels and the referring clinician have to be aware of the phenotypic target of the panel reflected in the selection of genes.

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Statement of Ethics

The study has been approved by the local ethical committees.

Disclosure Statement

The authors have no conflicts of interest to declare.

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