European Journal of Medical Genetics 64 (2021) 104363

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

Clinical next generation sequencing in developmental and epileptic encephalopathies: Diagnostic relevance of data re-analysis and variants re-interpretation

Valeria Salinas [a,b,*], Nerina Martínez [a], Josefina Pérez Maturo [a,b], Sergio A. Rodriguez-Quiroga [a], Lucia Zavala [a], Nancy Medina [a], Hernán Amartino [c], Ignacio Sfaello [d], Guillermo Agosta [e], Eva Maria Serafín [f], Dolores González Morón [a], Marcelo A. Kauffman [a,b,**], Patricia Vega [a]

[a] Neurogenetics Unit, Hospital JM Ramos Mejía, Buenos Aires, Argentina
[b] Precision Medicine and Clinical Genomics Group, Translational Medicine Research Institute-CONICET, Faculty of Biomedical Sciences, Universidad Austral, Buenos Aires, Argentina
[c] Paediatric Neurology Unit, Hospital Universitario Austral, Buenos Aires, Argentina
[d] CETES, Instituto de Neurología Infanto-Juvenil, Córdoba, Argentina
[e] Paediatric Neurology Unit, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina
[f] Genetic Unit, Hospital Aleman, Buenos Aires, Argentina

ARTICLE INFO

Keywords:
Variants of uncertain significance
Targeted gene panel sequencing
Whole exome sequencing
Pediatric epilepsy
Recessive epilepsy
Re-interpretation

ABSTRACT

Developmental and epileptic encephalopathies (DEE) are complex pediatric epilepsies, in which heterogeneous pathogenic factors play an important role. Next-generation-sequencing based tools have shown excellent effectiveness. The constant increase in the number of new genotype-phenotype associations suggests the periodic need for re-interpretation and re-analysis of genetic studies without positive results. In this study, we report the diagnostic utility of targeted gene panel sequencing and whole exome sequencing in 55 Argentine subjects with DEE, focusing on the utility of re-interpretation and re-analysis of undetermined and negative genetic diagnoses. The new information in biomedical literature and databases was used for the re-interpretation. For re-analysis, sequencing data processing was repeated using updated bioinformatics tools.

Initially, pathogenic variants were detected in 21 subjects (38%). After an average time of 29 months, 25% of the subjects without a genetic diagnosis were re-categorized as diagnosed. Finally, the overall diagnostic yield increased to 53% (29 subjects). In consequence of the re-interpretation and re-analysis, we identified novel variants in the genes: *CHD2, COL4A1, FOXG1, GABRA1, GRIN2B, HNRNPU, KCNQ2, MECP2, PCDH19, SCN1A, SCN2A, SCN8A, SLC6A1, STXBP1* and *WWOX*. Our results expand the diagnostic yield of this subgroup of infantile and childhood seizures and demonstrate the importance of re-evaluation of genetic tests in subjects without an identified causative etiology.

1. Introduction

Pediatric epilepsies are disorders that are differentiated by their etiology, age of presentation, seizure semiology, response to treatment, and prognosis (Scala et al., 2020). Including in this vast and heterogeneous group of disorders, Developmental and epileptic encephalopathies (DEE) are mainly characterized by severe seizures, poor response to drug treatment and a marked impact on neurodevelopmental prognosis (Kalser and Cross, 2018).

DEE are mainly can be attributed to alterations with underlying genetic factors, including brain malformations and metabolic diseases. In addition, this group of epilepsies can be caused by cerebrovascular

* Corresponding author. Neurogenetics Unit, Hospital JM Ramos Mejía, Buenos Aires, Argentina.
** Corresponding author. Neurogenetics Unit, Hospital JM Ramos Mejía. Buenos Aires, Argentina.
E-mail addresses: vmscmams@gmail.com (V. Salinas), nerina.martinez.nm@gmail.com (N. Martínez), josefinaperezmaturo@gmail.com (J.P. Maturo), sergiorodriguez@dr.com (S.A. Rodriguez-Quiroga), zavalalu@gmail.com (L. Zavala), genesisnan2020@gmail.com (N. Medina), hernan.amartino@gmail.com (H. Amartino), ignaciosfaello@gmail.com (I. Sfaello), guillermo.agosta@hospitalitaliano.org.ar (G. Agosta), estudiosgeneticosdraserafin@gmail.com (E.M. Serafín), doloresgm@gmail.com (D.G. Morón), marcelokauffman@gmail.com (M.A. Kauffman), neurogeninfantil@yahoo.com.ar (P. Vega).

https://doi.org/10.1016/j.ejmg.2021.104363
Received 11 December 2020; Received in revised form 24 September 2021; Accepted 8 October 2021
Available online 18 October 2021
1769-7212/Published by Elsevier Masson SAS.

disease, trauma, neurological infection (such as meningitis or encephalitis), neoplasia, autoimmune conditions, toxic insult, among others (Scheffer and Liao, 2020; Erkkinen and Berkowitz, 2019).

A wide variety of genes have been associated with these early epilepsies (Symonds and McTague, 2020), showing that several cellular functions may be involved in its pathogenesis. Nevertheless, frequently the genetic heterogeneity (different genes with a same or similar phenotype) or phenotypic heterogeneity (a gene with diverse phenotypes), make the diagnostic approach very difficult. Moreover, the growing evidence of the role of mosaic variants and the reduced penetrance or absence of symptoms observed in parents who carry causal variants of DEE in their children adds complexity to the diagnostic approach (Stosser et al., 2018; Sun et al., 2021).

Next generation sequencing (NGS) enabled the discovery of many genes that cause human disease. Consequently, NGS is widely used in the genetic diagnosis of epilepsies (Symonds and McTague, 2020; Stosser et al., 2018). The excellent cost-benefit ratio of NGS-based tools such as Targeted genes panel sequencing (TGPS) and Whole Exome Sequencing (WES) has facilitated their incorporation into the diagnostic workup in public and private neurogenetics services (Córdoba et al., 2018). The use of TGPS and WES has allowed the indication of optimized and personalized treatments to hundreds of patients (Bayat et al., 2021).

In recent years, WES and TGPS has increased the knowledge on the pathogenesis of DEEs, allowing the description of new phenotypic subtypes and the visualization of the complex genotype-phenotype associations that accompany this subgroup of pediatric epilepsies (Hebbar and Mefford, 2020). However, undetermined and negative genetic studies are often challenging.

It has been shown that re-interpretation and re-analysis of WES and TGPS data could improve the diagnostic yield in subjects without an identified etiology (Al-Nabhani et al., 2018; Trinh et al., 2019). Nevertheless, its impact has been evaluated in a low number of scientific reports (Li et al., 2019a).

In this study, we explore the diagnostic yield by using WES and TGPS to investigate peripheral blood samples from 55 DEE, including subjects with Dravet syndrome, *MECP2* and *PCDH19* related disorders, *STXBP1* encephalopathy, among others. In addition, we examine the clinical utility of re-interpretation and re-analysis of subjects without an initial genetic diagnosis (Fig. 1).

2. Material and methods

2.1. Subjects

Between 2016 and 2020, a total of 55 pediatric subjects (age range between 4 and 19 years) with DEE were selected for WES and TGPS in two medical centers from Argentina. These subjects were considered candidates for WES and TGPS due to a suspicion of neurogenetic diseases, including familial aggregation and the absence of non-genetic pathology that explains the phenotype. We recorded perinatal and family history, likely pattern of inheritance, disease progression characteristics, comorbidities, and studies performed before NGS testing. Before it was studied by NGS, most of the subjects underwent genetic analysis such as karyotype (100% of subjects) and/or chromosomal microarray (55% of subjects) without positive results.

All caregivers provided informed consent through a form approved by the Ethics Committee of the respective institutions. The informed consent included the option to receive incidental findings according to the American College of Medical Genetics (ACMG) recommendations when WES was performed.

2.2. Whole exome sequencing and targeted gene panel sequencing

Genomic DNA was isolated from peripheral blood with the use of commercial systems, following the manufacturer's instructions. This was kept anonymized. DNA sequencing libraries were constructed mostly by chemical fragmentation using commercial preparation kits. The most frequent methods for enrichment were capture-based target and amplicon-based target for exome and gene panel. NGS sequencing runs were made in Illumina systems (Illumina, INC) as an outsourced

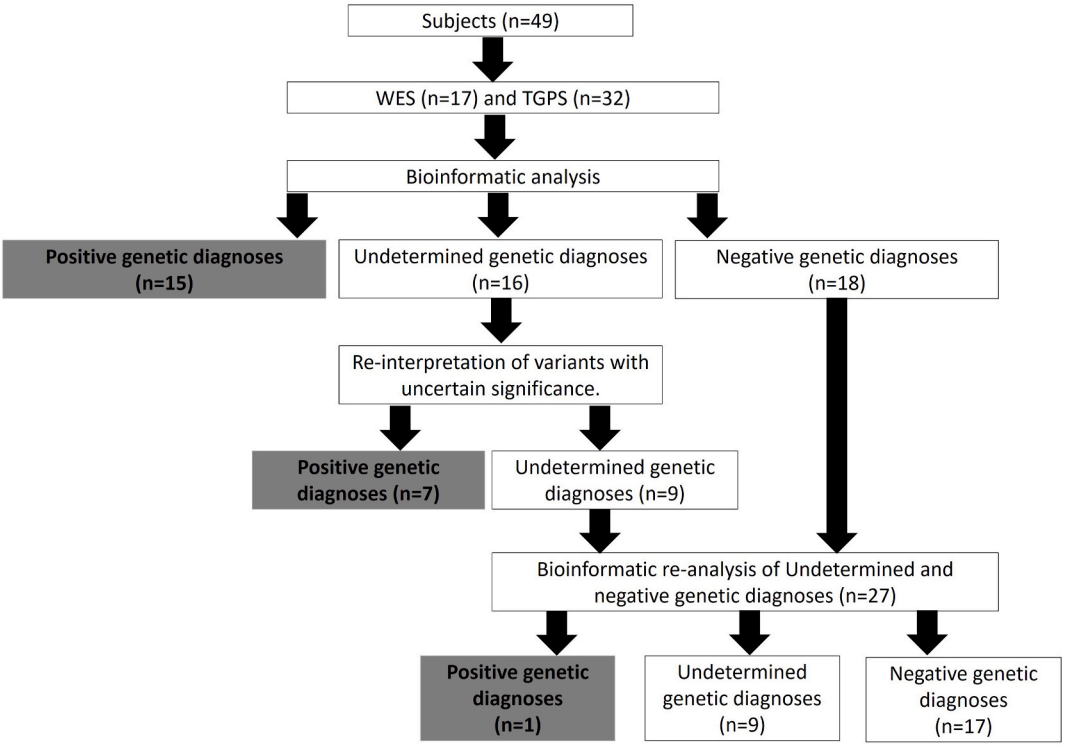

Fig. 1. Diagnosis flow chart in this study. The gray squares show the positive genetic diagnoses at each stage. Each patient with a positive genetic diagnosis received an updated clinical report and genetic counseling. The mean time for the re-categorization of subjects without genetic diagnosis as diagnosed was 29 months.

service. Detection of variants was possible with an average sequence coverage of more than 70X, with more than 97% of the target bases having at least 10X coverage. All standardized procedures were performed according to manufacturer's instructions, widely described in the literature (Margraf et al., 2011). Clinically relevant germinal variants, from the proband samples, were confirmed by Sanger sequencing. On the other hand, ultra-high depth NGS sequencing was used for the validation of a variant for which low allelic frequency (possible mosaicism) was observed in the results of WES and TGPS (Subject 10). Parental segregation by sanger sequencing was possible in 5 subjects. None of the patients underwent trio-WES or trio-TGPS.

2.3. Data analysis and annotation

Sequence data in FastQ format were aligned to the reference sequence of the human genome of the National Center for Biotechnology Information of the National Institutes of Health of the United States versions GRCh37 or GRCh38 using the Burrows-Wheeler Alignment Tool (BWA-MEM) (Li, 2013). Variant calls were generated using GATK3.6 or GATK4.1 haplotype caller following the so-called best practices (Van der Auwera et al., 2013). The output VCF file was annotated at various levels using Annovar (Wang et al., 2010), with information from several databases as previously described by our group (Córdoba et al., 2018).

We classified variants as pathogenic (P), probably pathogenic (LP), benign (B) and uncertain significance (VUS) according to the ACMG recommendations (Richards et al., 2015). In brief, ACMG categorizes variants considering their population frequency, molecular function, genetic position and effects predicted using in silico tools.

Joining variant characteristics and clinical features information, we classified each NGS study as **positive** if a pathogenic/likely pathogenic variant in a known disease gene was identified with compatible phenotypic and inheritance overlap; **undetermined** if a pathogenic/likely pathogenic variant in a putative candidate gene was identified without positive phenotypic and inheritance overlap; one pathogenic/likely pathogenic variant was identified with positive phenotypic overlap in a recessive disorder (unable to be detected in the other allele), one pathogenic/likely pathogenic variant was identified in a potential candidate gene not yet associated with disease; and **negative** in any other case. Incidental findings were informed according to ACMG recommendations. Counseling to subjects was performed by trained professionals.

2.4. Re-interpretation and re-analysis of subjects

Between April and September 2020, a new literature and database review was performed for all variants identified in subjects with undetermined genetic diagnoses (Fig. 1). In this revision, a phenotypic description and characterization of the respective variants was considered. The updated biomedical literature, medical databases and web search engines such as VarSome (The Human Genomic Variant Search Engine), PubMed and Google Scholar, were used for the re-interpretation of variants. Also, we performed a bioinformatic re-analysis of all subjects with a negative genetic diagnosis and in which the re-interpretation of variants was not enough for a reclassification to be genetically diagnosed (Fig. 1). This consisted of a new bioinformatic processing of the sequencing data, using updated genomic databases. We consider variant calling and filtering tools for the detection of mosaicism and germinal variants. Once a subject was reclassified, updated clinical reports were issued and received updated genetic counseling.

3. Results

Of the total number of subjects (n = 55), 31 females (56%) and 24 males (44%) were incorporated; 36 (65%) underwent TGPS and 19 (35%) were studied by WES (Table 1).

Detailed clinical information was available for 30% of the subjects. For 70% of the remaining subjects, we obtained the clinical diagnosis and basic phenotypic characteristics.

Criteria for a satisfied positive genetic diagnosis was present in 12/36 (30%) TGPS and 9/19 (50%) WES. So, the initial global diagnostic yield was 38% (Fig. 2). With 99% confidence, we did not find significant differences in diagnostic yield between TGPS and WES (Statistical power = 24,18% and p-value = 0, 7952) (Table 1, Fig. 2).

Studies classified as undetermined and negative genetic diagnosis correspond to 62% of our series. Re-interpretation and re-analysis steps were applied in 34 subjects (Fig. 1). Of all these subjects, 6 TGPS and 2 WES were re-categorized as positive genetic diagnoses (Table 1). In consequence, the global diagnostic yield improved from 38% (21 subjects) to 53% (29 subjects). The diagnostic yield of TGPS and WES increased to 50% (18 subjects) and 58% (11 subjects) respectively. With 99% confidence, we did not find significant differences in diagnostic yield between TGPS and WES (Statistical power = 9,09% and p-value = 0, 6542) (Fig. 2). The mean time elapsed from the original analysis to the reclassification was 29 months (standard deviation 12, 33).

Re-interpretation of variants in five subjects resulted from improved characterization of variants in genes known to cause DEE (Subjects 8,11,12,14 and 21). Furthermore, the emergence of additional clinical phenotypes attributed to variants initially indicated as undeterminate facilitated the genetic diagnosis of subjects 9 and 20 (Table 1).

Parental segregation studies were not possible for twenty-four subjects. However, the variants identified in these subjects were determined as the causal agent of their clinical characteristics by a multiaxial process in which the classification of the variant according to the ACMG, population allelic frequency and phenotype were considered.

Bioinformatic re-analysis and the publication of information that establishes a new genotype-phenotype association allowed the detection of a missense pathogenic variant in the *COL4A1* gene in a subject initially evaluated in early 2016 (Table 1). Since then, changes in this gene have been associated with a wide spectrum of neurological phenotypes and epilepsy secondary to brain cortical malformations, such as schizencephaly or polymicrogyria (Cavallin et al., 2018).

The final positive genetic diagnoses included 26 subjects with autosomal dominant inheritance, 2 autosomal recessive and 1 X-linked (Table 1). Missense, frameshift, nonsense and splice site alterations were the different variant types detected in our cohort (Table 1). In addition, a mosaic variant (observed in 66/128 or 52% of the readings) in the *PCDH19* gene was identified in subject 10. In our series, the genes most frequently identified as causative of DEEs were *SCN1A* (17%) and *SCN2A* (10%).

Novel variants (52%, 16/31) were identified, according to the reported in biomedical literature, in the genes: *CHD2, COL4A1, FOXG1, GABRA1, GRIN2B, HNRNPU, KCNQ2, MECP2, PCDH19, SCN1A, SCN2A, SCN8A, SLC6A1, STXBP1* and *WWOX*.

However, all the variants detected in *ARV1, CACNA1A, SCN1B* and *EPM2A* genes had already been reported in the literature (Fig. 3).

In order to identify the possible genetic mechanisms involved in DEE, we classify the altered genes according to their cellular function. In our series, 52% (16/31) of the variants identified are linked to channelopathies, suggesting that dysfunction of ion channels plays a key role in the pathophysiology of epileptic encephalopathies. Nuclear regulation was the second most affected cellular function (Fig. 4).

4. Discussion

In the last 10 years, the advent of massive sequencing techniques has revolutionized etiological diagnosis. Several hundred causative genes and their respective phenotypes have been reported in different genomic and clinical databases and the number keeps increasing daily (Symonds and McTague, 2020). This continuous growth of additional or new information about genotype-phenotype associations, allowed us to

Table 1
Subjects with molecular diagnosis by Targeted Gene Panel Sequencing (TGPS) and Whole Exome Sequencing (WES). Subjects with positive genetic diagnoses after re-interpretation (*) and re-analysis (**).

Subject	Sex	Clinical Diagnosis	NGS-based tool	Gene	Variant	Variant type	Inheritance pattern	ACMG classification	ACMG scoring	Literature (PMID)	Novel variant
1	F	Epileptic encephalopathy, early infantile, 6 (Dravet syndrome) (607208)	Single WES	*SCN1A*	NM_001165963: c.1178G>A:p.R393H	Missense	Autosomal dominant	Pathogenic	PM1 PM2 PM5 PP2 PP3 PP5	20431604	Not
2	F	Epileptic encephalopathy, early infantile, 6 (Dravet syndrome) (607208)	Single TGPS	*SCN1A*	NM_001165963: c.5180A>G:p.D1727G	Missense	Autosomal dominant	Pathogenic	PM1 PM2 PM5 PP2 PP3 PP5	Not previously reported	Yes
3	M	Epileptic encephalopathy, early infantile, 4 (612164)	Single TGPS	*STXBP1*	NM_001032221: c.1060T>C:p.C354R	Missense	Autosomal dominant	Likely Pathogenic	PM1 PM2 PM5 PP2 PP3 PP5	26514728	Not
4	M	Epileptic encephalopathy, early infantile, 6 (Dravet syndrome) (607208)	Single TGPS	*SCN1A*	NM_001165963:c. C677T:p.T226M	Missense	Autosomal dominant	Pathogenic	PM1 PM2 PM5 PP2 PP3 PP5	31257984	Not
5	F	Epileptic encephalopathy, early infantile, 7 (613720)	Single TGPS	*KCNQ2*	NM_004518:c.793G>A: p.A265T	Missense	Autosomal dominant	Pathogenic	PM1 PM2 PM5 PP2 PP3 PP5	27535030	Not
6	M	Epileptic encephalopathy, early infantile, 38 (617020)	Single WES	*ARV1*	NM_022786: c.294+1G>A NM_022786:c.565G>A: p.G189R	Splicing Missense	Autosomal recessive	Pathogenic Pathogenic	PVS1 PM2 PP3 PP5 PS1 PM1 PM2 PP3	27270415 27270415	Not Not
7	M	Epileptic encephalopathy, early infantile, 11 (613721)	Single WES	*SCN2A*	NM_001040143.1: c.4989delC:p. I1663Mfs*42	Frameshift	Autosomal dominant	Likely Pathogenic	PVS1 PM2	Not previously reported	Yes
8*	F	Epileptic encephalopathy, early infantile, 13 (614558)	Single TGPS	*SCN8A*	NM_014191: c.4477A>G:p.K1493E	Missense	Autosomal dominant	Uncertain Significance	PM2 PP2 PP3	Not previously reported	Yes
9*	F	Epileptic encephalopathy, early infantile, 42 (617106)	Single TGPS	*CACNA1A*	NM_001127221: c.653C>T:p.S218L	Missense	Autosomal dominant	Likely Pathogenic	PM2 PM5 PP2 PP3 PP5	21824570	Not
10	M	Epileptic encephalopathy, early infantile, 9 (300088)	Single TGPS	*PCDH19*	NM_001105243: c.1720G>T:p.E574X	Nonsense	X Linked **(Mosaicism seen in 66/ 128 or 52% of reads)**	Pathogenic	PVS1 PM2 PP3 PP5	Not previously reported	Yes
11*	F	Epileptic encephalopathy, early infantile, 11 (613721)	Single TGPS	*SCN2A*	NM_001040143: c.785T>C:p.F262S	Missense	Autosomal dominant	Likely Pathogenic	PM1 PM2 PP2 PP3	29314763	Not
12*	F	Epileptic encephalopathy, early infantile, 52 (617350)	Single TGPS	*SCN1B*	NM_001037:c.265C>T: p.R89C	Missense	Autosomal dominant	Uncertain Significance	PM1 PP2 PP3 BP1	31465153	Not
13	F	Epileptic encephalopathy, early infantile, 6 (Dravet syndrome) (607208)	Single WES	*SCN1A*	NM_006920: c.5314G>A:p.A1772T	Missense	Autosomal dominant	Pathogenic	PM1 PM2 PM5 PP2 PP3 PP5	30185235	Not
14*	F			*GABRA1*		Missense					Yes

(*continued on next page*)

Table 1 (*continued*)

Subject	Sex	Clinical Diagnosis	NGS-based tool	Gene	Variant	Variant type	Inheritance pattern	ACMG classification	ACMG scoring	Literature (PMID)	Novel variant
		Epileptic encephalopathy, early infantile, 19 (615744)	Single TGPS		NM_001127648: c.763A>T:p.I255F		Autosomal dominant	Likely Pathogenic	PM1 PM2 PP2 PP3	Not previously reported	
15	F	Rett Syndrome (312750)	Single WES	*MECP2*	NM_001110792.1: c.799C>T:p.R267T	Missense	Autosomal dominant	Pathogenic	PVS1 PP5 PM2 PP3	30185235	Not
16	F	Epileptic encephalopathy, early infantile, 7 (613720)	Single TGPS	*KCNQ2*	NM_172107.2: c.782T>G:p.F261C	Missense	Autosomal dominant	Likely Pathogenic	PM1 PM2 PP2 PP3	Not previously reported	Yes
17**	F	Epileptic encephalopathy (Not available)	Single TGPS	*COL4A1*	NM_001845.6: c.2069G>A:p.G690E	Missense	Autosomal dominant	Likely Pathogenic	PM1 PM2 PP2 PP3	Not previously reported	Yes
18	F	Epileptic encephalopathy, childhood-onset (615369)	Single TGPS	*CHD2*	NM_001271.4: c.2597C>A:p.S866T	Missense	Autosomal dominant	Pathogenic	PVS1 PM1 PP2 PP3	Not previously reported	Yes
19	M	Epileptic encephalopathy, early infantile, 54 (617391)	Single TGPS	*HNRNPU*	NM_031844.3: c.622C>T:p.Q208T	Missense	Autosomal dominant	Pathogenic	PVS1 PM2	Not previously reported	Yes
20*	F	Rett Syndrome (312750)	Single WES	*MECP2*	NM_001110792: c.710C>G:p.P237R	Missense	Autosomal dominant	Pathogenic	PSE PM1 PM2 PM5 PP2 PP3	Not previously reported	Yes
21*	M	Rett syndrome, congenital variant (613454)	Single WES	*FOXG1*	NM_005249: c.1007_1008insCCACC: p.M339Tfs*8	Frameshift	Autosomal dominant	Pathogenic	PVS1 PM2 PP3	Not previously reported	Yes
22	M	Epileptic encephalopathy, early infantile, 4 (612164)	Single WES	*STXBP1*	NM_001032221: c.1295T>A:p.I432K	Missense	Autosomal dominant	Uncertain Significance	PM2 PP2 PP3	Not previously reported	Yes
23	M	Epileptic encephalopathy, early infantile, 11 (613721)	Single WES	*SCN2A*	NM_001040143.2: c.788C>T:p.A263V	Missense	Autosomal dominant	Likely Pathogenic	PM1 PM2 PM5 PP2 PP3 PP5	26645390	Not
24	F	Epilepsy, progressive myoclonic 2A (254780)	Single TGPS	*EPM2A*	NM_001018041: c.94T>G:p.W32G	Missense	Autosomal dominant	Likely Pathogenic	PM1 PM2 PP2 PP3 PP5	12019207	Not
25	M	Myoclonic-atonic epilepsy (616421)	Single TGPS	*SLC6A1*	NM_001348250: c.518G>C:p.C173S	Missense	Autosomal dominant	Likely Pathogenic	PM1 PM2 PM5 PP2 PP3	Not previously reported	Yes
26	F	Epileptic encephalopathy, early infantile, 28 (616211)	Single TGPS	*WWOX*	NM_016373.4:c.231-3C>A NM_016373.4: c.107+1G>A	Splicing Splicing	Autosomal recessive	Uncertain Significance Pathogenic	PM2 PVS1 PM2 PP3 PP5	Not previously reported Not previously reported	Yes
27	F	Epileptic encephalopathy, early infantile, 6 (Dravet syndrome) (607208)	Single TGPS	*SCN1A*	NM_001165963.3: c.2665G>A:p.A889T	Missense	Autosomal dominant	Likely Pathogenic	PM1 PM2 PP2 PP3	Not previously reported	Yes
28	F	Epileptic encephalopathy, early infantile, 27 (616139)	Single WES	*GRIN2B*	NM_000834.5: c.2461G>T:p.V821F	Missense	Autosomal dominant	Likely Pathogenic	PM1 PM2 PP2 PP3	Not previously reported	Yes
29	F	Epileptic encephalopathy, early infantile, 27 (616139)	Single WES	*GRIN2B*	NM_000834.5: c.2065G>A:p.G689S	Missense	Autosomal dominant	Pathogenic	PM1 PM2 PP2 PP3 PP5	26350515	Not

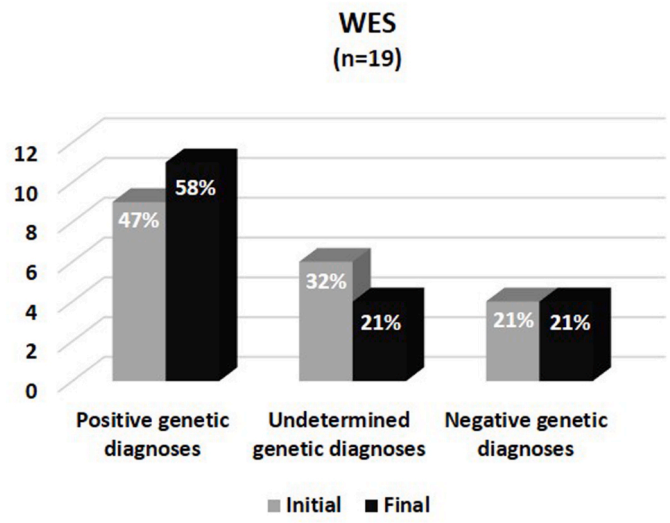

Fig. 2. Diagnostic yield initial and final (after re-interpretation and re-analysis) according to classification of patients diagnosed by Whole Exome Sequencing (WES) and Targeted Gene Panel Sequencing (TGPS) (Global), subjects diagnosed by WES and subjects diagnosed by TGPS.

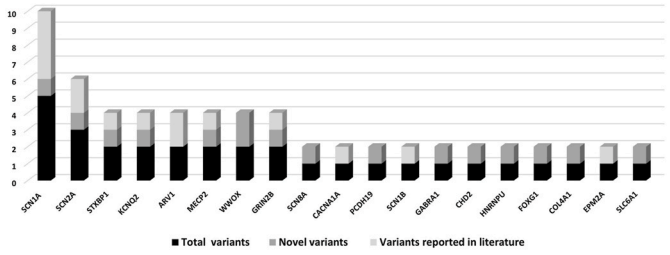

Fig. 3. Genes carrying variants identified in 51 subjects developmental and epileptic encephalopathies. The number of novel variants and previously reported in the literature is indicated.

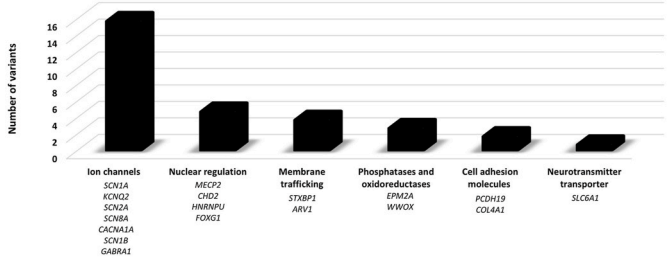

Fig. 4. Functional classification of genes that carry variants causal of developmental and epileptic encephalopathies.

increase to a diagnostic yield of 40% of positive diagnosis as a consequence of re-classification. Our re-evaluation showed a performance similar to that reported by Liu et al. for Mendelian disorders (Liu et al., 2019a).

The re-categorization of subjects without genetic diagnosis to diagnosed was in an average time of 29 months. Due to the small size of the series, it is not correct to suggest a frequency of re-analysis as a consequence of our results. However, other authors have suggested periods of time of 6–12 months for the reinterpretation of negative genetic studies in patients with epilepsy and intellectual disability (Li et al., 2019b; Nambot et al., 2018).

With a final global diagnostic rate of 53%, 26 subjects were not genetically diagnosed. It is possible that the pathogenic variant in some of these subjects is located in areas not covered by WES or TGPS sequencing, such as intronic regions. In addition, they are likely to correspond to genetic changes in genes that have not previously been associated with DEE or even a human disorder. On the other hand, rare copy number variants are the cause of approximately 14% of this type of pediatric seizure, but it is very difficult to detect them by WES and TGPS (Burdick et al., 2020; Hirabayashi et al., 2019).

Furthermore, we must consider that the limited clinical information available on some of the subjects belonging to our series probably prevented a superior improvement in the diagnostic yield. Consequently, it is essential to incorporate a detailed phenotypic description of all subjects in future studies.

Our series is heterogeneous and represents a wide variety of DEEs. We have included subjects with Dravet syndrome whose etiology is given in up to 96% of cases due to pathogenic variants in *SCN1A* (Nambot et al., 2018) and subjects with myoclonic-atonic seizures, in which the diagnostic yield drops to 14% depending on the series (Tang et al., 2020).

In our study, the final diagnostic yield for TGPS (50%) and WES (58%) was higher than that previously reported for DEEs (Gokben et al., 2017; Palmer et al., 2018). We did not find significant differences in diagnostic performance between TGPS and WES. However, the results of this comparison have limited statistical power and small sample size.

We identified a case of mosaicism in a boy in *PCDH19* gene, finding that it illustrates the usefulness of WES and TGPS in the recognition of this type of variants with low allelic frequency (Liu et al., 2019b). The discovery in the subject with PCDH19-related epilepsy are similar to previous reports of men carrying a somatic mutation in this X chromosome gene (Depienne et al., 2009; Romasko et al., 2018). The pathogenesis associated with these subjects would involve mechanisms of cellular interference at a locus where two alleles coexist due to the presence of mosaicism (Romasko et al., 2018).

Similarly to other reported series (Rochtus et al., 2020), *SCN1A* and *SCN2A* were the most frequently involved in this cohort. Noteworthy, there seems to be differences in the epileptic phenotype and drug responsiveness in these channelopathies dependent on the type of genetic variation. Gain-of-function pathogenetic variants in SCN2A have an average age of presentation before 3 months of age, whereas loss-of-function variants in SCN1A and SCN2A show a later onset after 3 months of age (Brunklaus and Lal, 2020). Similarly, the functional impact of the variants in SCN2A might determine the response of patients to drug therapy with sodium channel blockers (Wolff et al., 2017; Musto et al., 2020).

For the identification of sixteen pathogenic novel variants, it was necessary to establish a strategy for adequate interpretation of their pathogenic role. In general, this is challenging and requires a description of the phenotype in combination with different in silico prediction tools, clinical and functional data reported in the literature, population frequency, family history and parental segregation (Li et al., 2017). These steps are essential to increase the reliability of the NGS results. The detection of unreported variants in genes previously associated with DEE, strengthens rare genotype-phenotype associations and facilitates the molecular diagnosis of future patients.

In summary, we report findings that allow us to reinforce the efficacy of the use of NGS for the molecular approach of complex pediatric epilepsies. These data demonstrate the usefulness of the reinterpretation and re-analysis of undetermined and negative genetic studies, allowing to end the diagnostic odyssey of the patient and the family. Currently, many pharmacological treatments directed at the genetic etiology of epilepsies are under evaluation (Perucca and Perucca, 2019). Therefore, it is essential to continually establish new strategies to increase the diagnostic yield and open the doors of personalized medicine to the patients.

Ethics approval

This study was approved by the Institutional Ethics Committee of the Hospital JM Ramos Mejía of Buenos Aires, Argentina. All patients or, as appropriate, parents provided written informed consent for genetic analyses and use of their anonymized data. All experiments and methods were carried out in accordance with the relevant guidelines and approved. All clinical investigations have been conducted in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflicts of interest

Josefina Perez Maturo and Valeria Salinas have received scholarship support from Argentinean National Science Council (CONICET). Patricia Vega has received scholarship support from the Government of the Autonomous City of Buenos Aires. Marcelo A. Kauffman has received grant support from the Ministry of Health of Buenos Aires City, Argentinian National Science Council (CONICET) and Argentinean Ministry of Science and Technology. He serves as Associate Editor of Neurologia Argentina. The rest of the authors declare that they have no conflict of interest.

Funding

This study was funded by a grant from the Ministry of Science and Technology of Argentina.

Author statement

Valeria Salinas: Project administration, Visualization, Writing - Review & Editing, Writing - Original Draft, Formal analysis, Software, Methodology and Conceptualization. Nerina Martínez: Data Curation. Josefina Pérez Maturo: Data Curation. Sergio A. Rodriguez-Quiroga: Data Curation. Lucia Zavala: Data Curation. Nancy Medina: Data Curation. Hernán Amartino: Data Curation. Ignacio Sfaello: Data Curation. Guillermo Agosta: Data Curation. Eva Maria Serafín: Data Curation. Dolores González Morón: Funding acquisition, Project administration and Supervision. Marcelo A. Kauffman: Funding acquisition, Project administration, Supervision, Writing - Original Draft, Software and Conceptualization. Patricia Vega: Project administration, Supervision, Visualization, Writing - Review & Editing, Writing - Original Draft, Methodology and Conceptualization.

Acknowledgements

We thank the patients and families for their support and collaboration.

References

Al-Nabhani, M., Al-Rashdi, S., Al-Murshedi, F., Al-Kindi, A., Al-Thihli, K., Al-Saegh, A., Al-Futaisi, A., Al-Mamari, W., Zadjali, F., Al-Maawali, A., 2018. Reanalysis of exome sequencing data of intellectual disability samples: yields and benefits. Clin. Genet. 94, 495–501.

Bayat, A., Bayat, M., Rubboli, G., Møller, R.S., 2021. Epilepsy syndromes in the first year of life and usefulness of genetic testing for precision therapy. Genes 12 (7), 1051.

Brunklaus, A., Lal, D., 2020. Sodium channel epilepsies and neurodevelopmental disorders: from disease mechanisms to clinical application. Dev. Med. Child Neurol. 62, 784–792.

Burdick, K.J., Cogan, J.D., Rives, L.C., Robertson, A.K., Koziura, M.E., Brokamp, E., Duncan, L., Hannig, V., Pfotenhauer, J., Vanzo, R., Paul, M.S., Bican, A., Morgan, T., Duis, J., Newman, J.H., Hamid, R., Phillips, J.A., 2020. Undiagnosed Diseases Network. Limitations of exome sequencing in detecting rare and undiagnosed diseases. Am. J. Med. Genet. 182 (6), 1400–1406.

Cavallin, M., Mine, M., Philbert, M., Boddaert, N., Lepage, J.M., Coste, T., Lopez-Gonzalez, V., Sanchez-Soler, M.J., Ballesta-Martínez, M.J., Remerand, G., Pasquier, L., Guët, A., Chelly, J., Lascelles, K., Prieto-Morin, C., Kossorotoff, M., Tournier Lasserve, E., Bahi-Buisson, N., 2018. Further refinement of COL4A1 and COL4A2 related cortical malformations. Eur. J. Med. Genet. 61, 765–772.

Córdoba, M., Rodriguez-Quiroga, S.A., Vega, P.A., Salinas, V., Perez-Maturo, J., Amartino, H., Vásquez-Dusefante, C., Medina, N., González-Morón, D., Kauffman, M. A., 2018. Whole exome sequencing in neurogenetic odysseys: an effective, cost- and time-saving diagnostic approach. PLoS One 13 (2), e0191228.

Depienne, C., Bouteiller, D., Keren, B., Cheuret, E., Poirier, K., Trouillard, O., Benyahia, B., Quelin, C., Carpentier, W., Julia, S., Afenjar, A., Gautier, A., Rivier, F., Meyer, S., Berquin, P., Hélias, M., Py, I., Rivera, S., Bahi-Buisson, N., Gourfinkel-An, I., Leguern, E., 2009. Sporadic infantile epileptic encephalopathy caused by mutations in PCDH19 resembles Dravet syndrome but mainly affects females. PLoS Genet. 5 (2), e1000381.

Erkkinen, M.G., Berkowitz, A.L., 2019. A clinical approach to diagnosing encephalopathy. Am. J. Med. 132 (10), 1142–1147.

Gokben, S., Onay, H., Yilmaz, S., Atik, T., Serdaroglu, G., Tekin, H., Ozkinay, F., 2017. Targeted next generation sequencing: the diagnostic value in early-onset epileptic encephalopathy. Acta Neurol. Belg. 117, 131–138.

Hebbar, M., Mefford, H.C., 2020. Recent Advances in Epilepsy Genomics and Genetic Testing, p. F1000.

Hirabayashi, K., Uehara, D.T., Abe, H., Ishii, A., Moriyama, K., Hirose, S., Inazawa, J., 2019. Copy number variation analysis in 83 children with early-onset developmental and epileptic encephalopathy after targeted resequencing of a 109-epilepsy gene panel. J. Hum. Genet. 64, 1097–1106.

Kalser, J., Cross, J.H., 2018. The epileptic encephalopathy jungle - from Dr West to the concepts of aetiology-related and developmental encephalopathies. Curr. Opin. Neurol. 31, 216–222.

Li, H., 2013. Aligning Sequence Reads, Clone Sequences and Assembly Contigs with BWA-MEM.

Li, M.M., Datto, M., Duncavage, E.J., Kulkarni, S., Lindeman, N.I., Roy, S., Tsimberidou, A.M., Vnencak-Jones, C.L., Wolff, D.J., Younes, A., Nikiforova, M.N., 2017. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the association for molecular pathology, American society of clinical oncology, and College of American pathologists. J. Mol. Diagn. 19, 4–23.

Li, J., Gao, K., Yan, H., Xiangwei, W., Liu, N., Wang, T., Xu, H., Lin, Z., Xie, H., Wang, J., Wu, Y., Jiang, Y., 2019a. Reanalysis of whole exome sequencing data in patients with epilepsy and intellectual disability/mental retardation. Gene 700, 168–175.

Li, J., Gao, K., Yan, H., Xiangwei, W., Liu, N., Wang, T., Xu, H., Lin, Z., Xie, H., Wang, J., Wu, Y., Jiang, Y., 2019 Jun 5. Reanalysis of whole exome sequencing data in patients with epilepsy and intellectual disability/mental retardation. Gene 700, 168–175.

Liu, P., Meng, L., Normand, E.A., Xia, F., Song, X., Ghazi, A., Rosenfeld, J., Magoulas, P. L., Braxton, A., Ward, P., Dai, H., Yuan, B., Bi, W., Xiao, R., Wang, X., Chiang, T., Vetrini, F., He, W., Cheng, H., Dong, J., Gijavanekar, C., Benke, P.J., Bernstein, J.A., Eble, T., Eroglu, Y., Erwin, D., Escobar, L., Gibson, J.B., Gripp, K., Kleppe, S., Koenig, M.K., Lewis, A.M., Natowicz, M., Mancias, P., Minor, L., Scaglia, F., Schaaf, C.P., Streff, H., Vernon, H., Uhles, C.L., Zackai, E.H., Wu, N., Sutton, V.R., Beaudet, A.L., Muzny, D., Gibbs, R.A., Posey, J.E., Lalani, S., Shaw, C., Eng, C.M., Lupski, J.R., Yang, Y., 2019a. Reanalysis of clinical exome sequencing data. N. Engl. J. Med. 380 (25), 2478–2480.

Liu, A., Yang, X., Yang, X., Wu, Q., Zhang, J., Sun, D., Yang, Z., Jiang, Y., Wu, X., Wei, L., Zhang, Y., 2019b. Mosaicism and incomplete penetrance of PCDH19 mutations. J. Med. Genet. 56 (2), 81–88.

Margraf, R.L., Durtschi, J.D., Dames, S., Pattison, D.C., Stephens, J.E., Voelkerding, K.V., 2011. Variant identification in multi-sample pools by illumina genome analyzer sequencing. J. Biomol. Tech. 22 (2), 74–84.

Musto, E., Gardella, E., Møller, R.S., 2020 Jan. Recent advances in treatment of epilepsy-related sodium channelopathies. Eur. J. Paediatr. Neurol. 24, 123–128.

Nambot, S., Thevenon, J., Kuentz, P., Duffourd, Y., Tisserant, E., Bruel, A.L., Mosca-Boidron, A.L., Masurel-Paulet, A., Lehalle, D., Jean-Marçais, N., Lefebvre, M., Vabres, P., El Chehadeh-Djebbar, S., Philippe, C., Tran Mau-Them, F., St-Onge, J., Jouan, T., Chevarin, M., Poé, C., Carmignac, V., Vitobello, A., Callier, P., Rivière, J. B., Faivre, L., Thauvin-Robinet, C., Orphanomix Physicians' Group, 2018 Jun. Clinical whole-exome sequencing for the diagnosis of rare disorders with congenital anomalies and/or intellectual disability: substantial interest of prospective annual reanalysis. Genet. Med. 20 (6), 645–654.

Palmer, E.E., Schofield, D., Shrestha, R., Kandula, T., Macintosh, R., Lawson, J.A., Andrews, I., Sampaio, H., Johnson, A.M., Farrar, M.A., Cardamone, M., Mowat, D., Elakis, G., Lo, W., Zhu, Y., Ying, K., Morris, P., Tao, J., Dias, K.R., Buckley, M., Dinger, M.E., Cowley, M.J., Roscioli, T., Kirk, E.P., Bye, A., Sachdev, R.K., 2018. Integrating exome sequencing into a diagnostic pathway for epileptic encephalopathy: evidence of clinical utility and cost effectiveness. Mol Genet Genomic Med 6, 186–199.

Perucca, P., Perucca, E., 2019. Identifying mutations in epilepsy genes: impact on treatment selection. Epilepsy Res. 152, 18–30.

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W.W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H.L., 2015. 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. Genet. Med. 17, 405–424.

Rochtus, A., Olson, H.E., Smith, L., Keith, L.G., El Achkar, C., Taylor, A., Mahida, S., Park, M., Kelly, M., Shain, C., Rockowitz, S., Rosen Sheidley, B., Poduri, A., 2020. Genetic diagnoses in epilepsy: the impact of dynamic exome analysis in a pediatric cohort. Epilepsia 61, 249–258.

Romasko, E.J., DeChene, E.T., Balciuniene, J., Akgumus, G.T., Helbig, I., Tarpinian, J.M., Keena, B.A., Vogiatzi, M.G., Zackai, E.H., Izumi, K., Massey, S.L., Tayoun, A., 2018. PCDH19-related epilepsy in a male with Klinefelter syndrome: additional evidence supporting PCDH19 cellular interference disease mechanism. Epilepsy Res. 145, 89–92.

Scala, M., Bianchi, A., Bisulli, F., Coppola, A., Elia, M., Trivisano, M., Pruna, D., Pippucci, T., Canafoglia, L., Lattanzi, S., Franceschetti, S., Nobile, C., Gambardella, A., Michelucci, R., Zara, F., Striano, P., 2020. Advances in genetic testing and optimization of clinical management in children and adults with epilepsy. Expert Rev. Neurother. 20, 251–269.

Scheffer, I.E., Liao, J., 2020. Deciphering the concepts behind Epileptic encephalopathy and Developmental and epileptic encephalopathy. Eur. J. Paediatr. Neurol. 24, 11–14.

Stosser, M.B., Lindy, A.S., Butler, E., Retterer, K., Piccirillo-Stosser, C.M., Richard, G., McKnight, D.A., 2018. High frequency of mosaic pathogenic variants in genes causing epilepsy-related neurodevelopmental disorders. Genet. Med. 20, 403–410.

Sun, S., Li, X., Song, J., Li, Y., Xu, L., Xia, B., Hua, Y., Zhu, L., Wang, J., 2021. Analysis of clinical phenotype and SCN1A gene variant in a pedigree affected with genetic epilepsy with febrile seizures. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 10 (8), 745–748, 38.

Symonds, J.D., McTague, A., 2020. Epilepsy and developmental disorders: next generation sequencing in the clinic. Eur. J. Paediatr. Neurol. 24, 15–23.

Tang, S., Addis, L., Smith, A., Topp, S.D., Pendziwiat, M., Mei, D., Parker, A., Agrawal, S., Hughes, E., Lascelles, K., Williams, R.E., Fallon, P., Robinson, R., Cross, H.J., Hedderly, T., Eltze, C., Kerr, T., Desurkar, A., Hussain, N., Kinali, M., Bagnasco, I., Vassallo, G., Whitehouse, W., Goyal, S., Absoud, M., , EuroEPINOMICS-RES Consortium, Møller, R.S., Helbig, I., Weber, Y.G., Marini, C., Guerrini, R., Simpson, M.A., Pal, D.K., 2020. Phenotypic and genetic spectrum of epilepsy with myoclonic atonic seizures. Epilepsia 61, 995–1007.

Trinh, J., Kandaswamy, K.K., Werber, M., Weiss, M.E.R., Oprea, G., Kishore, S., Lohmann, K., Rolfs, A., 2019. Novel pathogenic variants and multiple molecular diagnoses in neurodevelopmental disorders. J. Neurodev. Disord. 11 (1), 11.

Van der Auwera, G.A., Carneiro, M.O., Hartl, C., Poplin, R., Del Angel, G., Levy-Moonshine, A., Jordan, T., Shakir, K., Roazen, D., Thibault, J., Banks, E., Garimella, K.V., Altshuler, D., Gabriel, S., DePristo, M.A., 2013. From FastQ data to high confidence variant calls: the genome analysis toolkit best practices pipeline. Current protocols in bioinformatics 43 (1110), 11.10.1-11.10.33.

Wang, K., Li, M., Hakonarson, H., 2010. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 38 (16), e164.

Wolff, M., Johannesen, K.M., Hedrich, U.B.S., Masnada, S., Rubboli, G., Gardella, E., Lesca, G., Ville, D., Milh, M., Villard, L., Afenjar, A., Chantot-Bastaraud, S., Mignot, C., Lardennois, C., Nava, C., Schwarz, N., Gérard, M., Perrin, L., Doummar, D., Auvin, S., Miranda, M.J., Hempel, M., Brilstra, E., Knoers, N., Verbeek, N., van Kempen, M., Braun, K.P., Mancini, G., Biskup, S., Hörtnagel, K., Döcker, M., Bast, T., Loddenkemper, T., Wong-Kisiel, L., Baumeister, F.M., Fazeli, W., Striano, P., Dilena, R., Fontana, E., Zara, F., Kurlemann, G., Klepper, J., Thoene, J.G., Arndt, D.H., Deconinck, N., Schmitt-Mechelke, T., Maier, O., Muhle, H., Wical, B., Finetti, C., Brückner, R., Pietz, J., Golla, G., Jillella, D., Linnet, K.M., Charles, P., Moog, U., Öiglane-Shlik, E., Mantovani, J.F., Park, K., Deprez, M., Lederer, D., Mary, S., Scalais, E., Selim, L., Van Coster, R., Lagae, L., Nikanorova, M., Hjalgrim, H., Korenke, G.C., Trivisano, M., Specchio, N., Ceulemans, B., Dorn, T., Helbig, K.L., Hardies, K., Stamberger, H., de Jonghe, P., Weckhuysen, S., Lemke, J.R., Krägeloh-Mann, I., Helbig, I., Kluger, G., Lerche, H., Møller, R.S., 2017. Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. Brain 140, 1316–1336.