**ORIGIN AL AR TICLE**

Customized targeted massively parallel sequencing enables the identification of novel pathogenic variants in Tunisian patients with developmental and epileptic encephalopathy

**Abstract**

**Objective:** To develop a high-throughput sequencing panel for the diagnosis of developmental and epileptic encephalopathy in Tunisia and to clarify the fre- quency of disease-causing genes in this region.

**Methods:** We developed a custom panel for next-generation sequencing of the coding sequences of 116 genes in individuals with developmental and epileptic encephalopathy from the Tunisian population. Segregation analyses and in silico studies have been conducted to assess the identified variants' pathogenicity.

**Results:** We report 12 pathogenic variants in *SCN1A*, *CHD2*, *CDKL5*, *SZT2*,

*KCNT1*, *GNAO1*, *PCDH19*, *MECP2*, *GRIN2A*, and *SYNGAP1* in patients with developmental and epileptic encephalopathy. Five of these variants are novel: “c.149delA, p.(Asn50MetfsTer26)” in *CDKL5*; “c.3616C > T, p.(Arg1206Ter)” in *SZT2*; “c.111\_113del, p.(Leu39del)” in *GNAO1*; “c.1435G>C, p.(Asp479His)” in *PCDH19;* and “c.2143delC, p.(Arg716GlyfsTer10)” in *SYNGAP1*. Additionally, for four of our patients, the genetic result facilitated the choice of the appropriate treatment.

**Significance:** This is the first report of a custom gene panel to identify ge-

netic variants implicated in developmental and epileptic encephalopathy in the Tunisian population as well as the North African region (Tunisia, Egypt, Libya, Algeria, Morocco) with a diagnostic rate of 30%. This high-throughput sequenc- ing panel has considerably improved the rate of positive diagnosis of develop- mental and epileptic encephalopathy in the Tunisian population, which was less than 15% using Sanger sequencing. The benefit of genetic testing in these pa- tients was approved by both physicians and parents.

**KEYWORDS**

developmental and epileptic encephalopathy, diagnosis, panel, sequencing, variant

# | INTRODUCTION

Epilepsy is a common neurological disease with 50 mil- lion affected individuals worldwide. As a result, it is among the most frequent neurological diseases globally. Relying on the World Health Organization, approxi- mately three-quarters of them live in low- and middle- income countries. Additionally, the premature death risk in people with epilepsy amounts to three times higher than for the general population.[1](#_bookmark6) Developmental and epileptic encephalopathy (DEE) corresponds to a heterogeneous group of epileptic syndromes marked by early-onset, refractory seizures that also take place within the framework of developmental regression.[2](#_bookmark7) According to the ILAE classification of epileptic syn- dromes, DEE encompasses several clinically definable epilepsy syndromes including Dravet syndrome (DS), infantile epileptic spasms syndrome (IESS), Lennox- Gastaut syndrome (LGS), and epilepsy of infancy with migrating focal seizures (EIMFS).[2–4](#_bookmark7) Etiologies of DEE are variable. With the advances in sequencing meth- ods, genetic etiologies become more and more frequent and reach more than 50% of patients with early-onset DEE.[5](#_bookmark8) In Tunisia, DEE constitutes a significant burden for the family and the healthcare system. It represents 43% of the epileptic syndrome in the Sfax Department of Child Neurology. In Tunisia, as well as in North Africa, data are scarce regarding the genetic basis of epilepsy. In fact, in the whole African continent, only one study has recently been published. It was concerned with the development of a DEE panel in the South African re- gion.[6](#_bookmark9) Therefore, exploration of the etiology of DEE and the early diagnosis of causal variants by next-generation sequencing (NGS) assist to a great extent in terms of al- leviating social and economic problems. Scrutinizing through literature, multiple research works tackled the use of gene panels in epilepsy with variable diagnostic yields.[7](#_bookmark10) So far, this variability made it difficult to identify an appropriate gene pool whose screening might enable accurate diagnosis and generate higher diagnostic yields in people displaying heterogeneous epilepsy pheno- types. From this perspective, wide-ranging approaches, such as whole exome sequencing (WES) or whole ge- nome sequencing (WGS), are basically adopted as alter- native genetic testing methods. Yet, the interpretation of gene variants with WES and WGS is not only time-con- suming but also a more sophisticated task. Currently, panel-based sequencing is still preferred in certain clini- cal centers, owing to its depth of coverage, speed of data analysis as well as cost saving.[8](#_bookmark11)

In our study, we built up a comprehensive NGS panel interrogating 116 genes implicated in DEE. The panel covered the coding exons and the exon-intron junctions,

providing a high-throughput assay. Our basic objective is to clarify the frequency of disease-causing genes among the Tunisian population.

# | PATIENTS AND METHODS

## | Subjects and sample preparation

We collected 40 Tunisian children from the region of Sfax and southern Tunisia. Recruitment of patients was under- taken between 2020 and 2022 in the Department of Child Neurology of Hedi Chaker Hospital in Sfax. All patients were examined and diagnosed by a pediatric neurologist. Clinical data, including age at the onset, frequency, and type of seizures, as well as the presence of developmental delay or regression, acquired or congenital microcephaly, abnor- mal movements or stereotypies, and dysmorphic syndrome, were analyzed. Seizure types and epileptic syndromes have been diagnosed and classified relying on International League Against Epilepsy classification.[2–4](#_bookmark7)

Whole blood was collected from all the patients. Samples from additional family members were invested whenever possible to conduct segregation analysis of the sequence variants identified in the index patient. We ex- tracted DNA from all samples through the use of the phe- nol-chloroform standard method.

## | Capture design and targeted next-generation sequencing

We designed a hybridization-based multi-disease gene panel using Design Studio which is a web application from Illumina to devise a custom target enrichment library design.

**Key Points**

* This is the first report of a custom gene panel to identify genetic variants implicated in develop- mental and epileptic encephalopathy in North Africa.
* 12 pathogenic variants were reported for the first time in the Tunisian population.
* The high-throughput sequencing panel has considerably improved the rate of positive diag- nosis of developmental and epileptic encepha- lopathy in Tunisia.
* For four of our patients, the genetic result facil- itated the choice of the appropriate treatment.

The design rested on GRCh37/hg19 reference sequences, with target sources obtained from the RefSeq database. All coding exons in this custom design were targeted includ- ing 25 bp of the flanking intronic sequence of 116 genes. The criteria for including a gene on the panel were that it should have been reported more than once in patients with epilepsy. All selected genes are included in Table [S1](#_bookmark65). Most of these genes have an autosomal dominant or an autosomal recessive inheritance mode (Figure [S1](#_bookmark64)). Libraries were pre- pared relying on the “Illumina DNA Prep with Enrichment sample preparation reference guide”. The libraries were se- quenced on the Miseq system (Illumina).

## | Bioinformatic pipeline

We adjusted MiSeq Reporter software settings (Illumina) to generate VCF files for index reads. VarAFT was used for variant annotation and filtering.[9](#_bookmark12) The mean sequenc- ing depth is 165× (Min = 109×, Max = 214×). Variants with minor allelic frequency (MAF) < 0.1% were re- tained. Nonsense variants and small deletions or inser- tions inducing a frameshift of the coding sequence were regarded as the most harmful, as they necessarily altered the amino-acid sequence of the protein. We also examined the pathogenicity of the different variants in accordance with ACMG (American College of Medical Genetics and Genomics) standards and guidelines.[10](#_bookmark13) Variants were clas- sified into five types, namely “Benign,” “Likely Benign,” “Pathogenic,” “likely pathogenic,” and “Uncertain sig- nificance.” Moreover, AF in the genome Aggregation Database (gnomAD) ([https://gnomad.broadinstitute.](https://gnomad.broadinstitute.org/) [org/](https://gnomad.broadinstitute.org/)) was invested to assess the variant's frequency.

We estimated the pathogenicity of missense variants through the use of SIFT and PROVEAN algorithms.[11](#_bookmark14) Additionally, we specified the pathogenicity of the sub- stitution variants by Mutation Taster.[12](#_bookmark15) To check if the sites of the variants are conserved, we used PhyloP100way whose scores rely on many alignments of 99 vertebrate genome sequences to the human genome. The higher the score, the more conserved the site.

## | Validation by Sanger sequencing

We analyzed variants suspected to be pathogenic using Sanger sequencing. We amplified DNA fragments in- volving the variants by PCR with specific primers and we sequenced them on both strands using the Big Dye

3.1 Terminator Sequencing Kit. We analyzed purified sequence products on a 3100 ABI instrument (Applied Biosystems). We conducted a segregation analysis in cases where DNA samples of relatives were obtainable.

1. | **RESULTS**

## | Clinical findings

We collected 40 Tunisian children with DEE. Pathogenic variants were identified per patient and their respective clinical presentations are illustrated in Table [1](#_bookmark3).

## | Genetic findings

A total of 12 variants were identified and predicted to be pathogenic or likely pathogenic according to ACMG clas- sification[10](#_bookmark13) (Table [S4](#_bookmark65)) (Two frameshift, five missense, four nonsense, and one in-frame variants). The commonest gene in which positive findings were identified was *SCN1A* (3 patients). Additionally, variants were identified in *CDKL5*, *GNAO1*, *KCNT1*, *CHD2*, *PCDH19*, *SZT2*, *MECP2*, *GRIN2A*,

and *SYNGAP1*. Among these variants, five are novel and seven were previously reported in the literature. Table [2](#_bookmark4) shows the number of pathogenic variants detected in the study as well as the results of the software's predictions.

## | Treatment adaptations offered to patients after the genetic diagnosis

According to the World Health Organization, it is recorded that up to 70% of people affected with epilepsy can live seizure-free if they are properly diagnosed and treated.[1](#_bookmark6) In the current study, 12 pathogenic or likely pathogenic vari- ants were identified. Identification of a specific underlying genetic variant can guide precision medicine in terms of preventing paradoxical aggravation of certain epilepsies. In fact, it is crucial to manage seizures carefully so as to shun disability and injuries and minimize the risk of life- threatening complications, such as sudden unexpected death in epilepsy and status epilepticus. In this study, four patients benefited from treatments after genetic diagno- sis. The ages of these patients in the study range from 4 to 12 years and the ages of the first symptoms range from 1.3 to 8 months (Table [1](#_bookmark3)). As far as DS is concerned, its early suspicion by means of *SCN1A* loss of function variants' identification may be extremely beneficial. Sodium chan- nel-blocking drugs need to be avoided, as they may even aggravate the seizures or are likely to be ineffective.[36,37](#_bookmark34) In such cases, alternative treatments involve benzodiaz- epines, valproate, stiripentol, cannabinoids, fenfluramine, and the ketogenic diet.[37–41](#_bookmark35) For our patients (SEED.0009 and SEED.0198) carrying “c.3094G > T, p.(Glu1032Ter)” and “c.1837C > T, p.(Arg613Ter)” variants in *SCN1A*, these alternative treatments were prescribed. In this re- spect, it was reported that Levetiracetam corresponds to a

**TABLE 1** Clinical features of the patients in the cohort.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient** | **Variant** | **Sex** | **Neurodevelopment history** | **Consanguinity** | **Family history** | **Age at seizure onset (month)** | **Age at the study (years)** |
| SEED.0009 | *SCN1A*: c.3094G > T  p.Glu1032Ter | F | Motor and cognitive delay | No | Sister: dysmorphic features  Congenital cardiopathy | 4 | 4 |
| SEED.0020 | *GNAO1*:  c.111-113del p.Leu39del | M | Motor and cognitive delay | No | – | 1 | 6 |
| SEED.0021 | *CDKL5*:  c.149delA p.Asn50MetfsTer26 | F | Motor and cognitive delay | Yes | – | 1.3 | 10 |
| SEED.0061 | *KCNT1*: c.2714G > A  p.Arg905Gln | M | Motor and cognitive delay | No | – | 11 | 17 |
| SEED.0062 | *CHD2*: c.2698C > T  p.Arg900Ter | F | Motor and cognitive delay | Yes | Autism (cousin) | 38 | 10 |
| SEED.0074 | *PCDH19*: c.1435G > C  p.Asp479His | F | Motor and cognitive delay | No | – | 8 | 7 |
| SEED.0093 | *SZT2*: c.3616C > T  p.Arg1206Ter | M | Motor and cognitive delay | Yes | – | 2 | 5 |
| SEED.0113 | *MECP2*: c.433C > T  p.Arg145Cys | F | Motor and cognitive delay | No | – | 18 | 12 |
| SEED.0139 | *SCN1A*: c.4756G > A  p.Gly1586Arg | M | Motor and cognitive delay | Yes | – | 3 | 19 |
| SEED.0151 | *SYNGAP1*:  c.2143delC p.Arg716GlyfsTer10 | F | Motor and cognitive delay | Yes | Febrile seizures: mother's cousins | 16 | 17 |
| SEED.0158 | *GRIN2A*: c.1510C > T  p.Arg504Trp | F | Motor and cognitive delay | No | – | 40 | 11 |
| SEED.0198 | *SCN1A*: c.1837C > T  p.Arg613Ter | F | Motor and cognitive delay | No | – | 6 | 12 |

Abbreviations: DS, Dravet syndrome; EIDEE, early infantile developmental and epileptic encephalopathy; F, female; IESS, infantile epileptic spasm syndrome; LGS, Lennox-Gastaut syndrome; M, male; PCDH19-Clustering Epilepsy, procadherin 19 clustering epilepsy; SHE, sleep-related hypermotor epilepsy.

powerful and reliable therapy for females with *PCDH1*9- Girls clustering epilepsy and has to be considered early in the management of the highly refractory clusters of sei- zures that characterize this genetic disease.[42](#_bookmark36) Moreover, Ganaxolone was reported to significantly reduce the

frequency of CDKL5 Deficiency Disorder-associated sei- zures.[43](#_bookmark37) For these reasons, SEED.0074 and SEED.0021 benefited from Levetiracetam and Ganaxolone, respec- tively. Now, we are monitoring the effect of treatments prescribed to patients.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Seizure type** | **Interictal EEG** | **Seizure evolution** | **Other clinical features** | **Epileptic syndrome** | **Brain MRI** | **Treatment adaptations offered after the genetic diagnosis** |
| Febrile Focal clonic | Normal | Febrile status epilepticus | Intellectual deficiency with poor expressive language | DS | Normal | Benzodiazepines, valproate, cannabinoids |
| Tonic generalized | Normal | Focal clonic | Severe intellectual deficiency | EIDEE | Cortical  atrophy | – |
| Myoclonus  generalized | Multifocal  discharges | Spasms | Severe intellectual deficiency | IESS | Normal | Ganaxolone |
| Tonic | Multifocal  discharges | Persisting | Severe intellectual deficiency with autism spectrum disorder | SHE | Normal | – |
| Tonic | Fast rhythms/ spike-waves | No seizures | Severe intellectual deficiency | LGS | Normal | – |
| Tonic (clusters) | Normal | Improvement | Severe intellectual deficiency with autism spectrum disorder | PCDH19-  clustering epilepsy | Normal | Levetiracetam |
| Tonic/clonic | Multifocal  discharges | Persisting | -Severe intellectual deficiency  -Dysmorphic features  -Global hypotonia, non-ambulatory | Infantile DEE | Not done | – |
| Generalized tonic during sleep | Centro-temporal spikes | Persisting | -Severe intellectual deficiency with autism spectrum disorder  -Hands washing  -Jovial mood | Infantile DEE | Normal | – |
| Clonic | Normal | Persisting | Severe intellectual deficiency | DEE with  fever- sensitive epilepsy | Hippocampal dysgeusia | – |
| Febrile seizures Typical absences Focal tonic | Multifocal spikes | Improved under Lamotrigine | Severe intellectual deficiency | Infantile DEE | Normal | – |
| Generalized tonic | Centro-temporal spikes activated with sleep | Persisting | Mild intellectual deficiency | DEE-SWAS | Normal | – |
| Clonic/tonic/ Normal Febrile and Mild intellectual DS Normal Benzodiazepines, myoclonic afebrile deficiency with valproate,  febrile learning difficulties cannabinoids | | | | | | |

# | DISCUSSION

In Tunisia, the analysis of DEE remains confined to a few cases based on candidate gene approach or clinical exome sequencing instead of targeted sequencing.[44–53](#_bookmark38) Yet, in

terms of DEE for which many candidate genes were re- ported, investing in targeted sequencing may be an op- timal method for choice as this approach facilitates the analysis of generated sequencing data, reduces sequenc- ing cost, increases sequencing depth, and does not include

**TABLE 2** Genetic characteristic of variants identified in this study.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient** | **Gene** | **Transcript** | **Exon** | **cDNA** | **Variant** | **Genotype** | **Variant type** |
| SEED.0009 | *SCN1A* | NM\_[001353948.2](https://varsome.com/transcript/hg19/NM_001353948.2) | 17 | c.3094G > T | p.Glu1032Ter | Het | Nonsense |
| SEED.0139 |  | NM\_[001353948.2](https://varsome.com/transcript/hg19/NM_001353948.2) | 26 | c.4756G > A | p.Gly1586Arg | Het | Missense |
| SEED.0198 |  | NM\_[001353948.2](https://varsome.com/transcript/hg19/NM_001353948.2) | 12 | c.1837C > T | p.Arg613Ter | Het | Nonsense |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| SEED.0021 | *CDKL5* | NM\_003159.2 | 4 | c.149delA | p.Asn50MetfsTer26 | Het | Frameshift |
| SEED.0020 | *GNAO1* | NM\_138736.3 | 1 | c.111\_113del | p.Leu39del | Het | In-frame |
| SEED.0061 | *KCNT1* | NM\_001272003.2 | 24 | c.2714G > A | p.Arg905Gln | Het | Missense |
| SEED.0062 | *CHD2* | NM\_001271.4 | 21 | c.2698C > T | p.Arg900Ter | Het | Nonsense |
| SEED.0074 | *PCDH19* | NM\_001105243.2 | 1 | c.1435G > C | p.Asp479His | Het | Missense |
| SEED.0093 | *SZT2* | NM\_015284.4 | 25 | c.3616C > T | p.Arg1206Ter | Hom | Nonsense |
| SEED.0113 | *MECP2* | NM\_001110792 | 3 | c.433C > T | p.Arg145Cys | Het | Missense |
| SEED.0151 | *SYNGAP1* | NM\_001130066 | 13 | c.2143delC | p.Arg716GlyfsTer10 | Het | Frameshift |
| SEED.0158 | *GRIN2A* | NM\_001134407 | 7 | c.1510C > T | p.Arg504Trp | Het | Missense |

Abbreviations: D, damaging; DC, disease causing; Het, heterozygous; Hom, homozygous; LP, likely pathogenic; P, pathogenic; S, supporting; U, uncertain; US, uncertain significance; VNF, variant not found; VS, very strong.

any ethical concerns in terms of the return results. This report corresponds to the first one tackling a custom gene panel to identify genetic variants implicated in DEE in the Tunisian population with a positive diagnostic rate of 30% (12/40). This diagnosis rate was less than 15% before the development of the panel.

## | Expanding the spectrum of DEE variants in Tunisian patients

In this research work, we successfully identified five novel pathogenic variants and seven preceding reported patho- genic variants in the Tunisian population for the first time.

Variants in *SCN1A* were reported previously in Tunisian patients with DS or generalized epilepsy with febrile seizures plus (GEFS+).[47,49,50](#_bookmark40) However, this is the first report of “p.(Glu1032Ter),” “p.(Arg613Ter),” and

“p.(Gly1586Arg)” variants in Tunisia. According to the literature, the “p.(Glu1032Ter)” variant in *SCN1A* is re- ported twice in DS patients,[13,14](#_bookmark16) and “p.(Arg613Ter)” vari- ant is reported several times in patients with DS[17,18,20–22,54](#_bookmark20) (Table [S2](#_bookmark65)). Our patients (SEED.0009 and SEED.0198) present also a DS (Table [S2](#_bookmark65)), which goes in good consis- tency with previous studies showing that this syndrome is associated with loss of function variants.[55](#_bookmark41) The “p.(Gl- y1586Arg)” variant was previously reported twice in an Italian and a Turkish patient with DS and unclassified epilepsy, respectively[15,16](#_bookmark18) (Table [S2](#_bookmark65)). However, our patient presents DEE with fever-sensitive epilepsy.

Additionally, the “p.(Arg905Gln)” variant is detected in *KCNT1*. This variant was recorded previously in affected persons with DEE,[23,30](#_bookmark26) childhood-onset epilepsy,[14,29](#_bookmark17) EIMFS with or without IESS,[24,26–28,31,32](#_bookmark27) autosomal domi- nant nocturnal frontal lobe epilepsy (ADNFLE),[56](#_bookmark42) autoso- mal dominant forms of sleep-related hypermotor epilepsy

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Transmission** | **Mutation taster** | **SIFT** | **Provean** | **AF in gnomAD exomes v 2.1.1 (global)** | **PhyloP100 conservation score** | **Reported variant/ reference** | **ACMG**  **classification** |
| Not available | DC | – | – | VNF | 4.88 | [[13](#_bookmark16)]  [[14](#_bookmark17)] | LP |
| De Novo | DC | D | D | VNF | 3.796 | [[15](#_bookmark18)]  [[16](#_bookmark19)] | LP |
| Not available | - | – | – | VNF | 0.45 | [[17](#_bookmark20)]  [[18](#_bookmark21)]  [[19](#_bookmark22)]  [[20](#_bookmark23)]  [[21](#_bookmark24)]  [[22](#_bookmark25)] | P |
| De Novo | – | – | – | VNF | 9.343 | Novel | LP |
| Not available | – | – | – | VNF | 7.53 | Novel | LP |
| De Novo | DC | D | D | VNF | 9.577 | [[14,23](#_bookmark17)]  [[24,25](#_bookmark27)]  [[26,27](#_bookmark29)]  [[28,29](#_bookmark30)]  [[30,31](#_bookmark31)]  [[32,33](#_bookmark32)] | LP |
| De Novo | U | – | – | VNF | 3.97 | Novel | P |
| Maternal | DC | D | D | VNF | 7.82 | Novel | P |
| Inherited from parents | DC | – | – | 0.0000279 | 2.57 | Novel | LP |
| De Novo | U | D | D | VNF | 9.29 | Reported 141 times ([https://varsome.](https://varsome.com/) [com](https://varsome.com/)) | P |
| De Novo | – | – | – | VNF | 5.61 | Novel | LP |
| De Novo | B | D | D | VNF | 2.59 | [[34,35](#_bookmark33)] | P |

(ADSHE),[25,33](#_bookmark28) and early-onset epileptic encephalopathy (EOEE)[26](#_bookmark29) in many populations. Referring to our patient (SEED.0061), the “p.(Arg905Gln)” variant is responsible for sleep-related hypermotor epilepsy (Table [S2](#_bookmark65)). The co- existence of different phenotypes for the same genetic vari- ation is indicative that possibly genetic or environmental modifiers exist, which suggests the need for extended re- search into gene variants.

We equally identified a truncating variant “p.(Arg900Ter)” in *CHD2* causing LGS in the patient. This variant has never been described in the literature but it has been reported in the ClinVar database as being involved in DEE 94. So far, according to the Human Gene Mutation Database, 86 disease-causing variants have been reported in *CHD2*. The described patients presented epileptic en- cephalopathy, intellectual disability ranging in severity, autism spectrum disorder, myoclonic seizures, status epi- lepticus, and ataxia.[57](#_bookmark43) The majority of pathogenic variants

are truncating (>72%).[58](#_bookmark44) However, in spite of the high number of previously reported *CHD2* pathogenic or likely pathogenic variants, no clear genotype-phenotype correla- tion was found. Our study characterizes the tenth patient worldwide carrying a variant in *CHD2* with LGS as a phe- notype.[58,59](#_bookmark44) The identification of variants implicated in the same phenotype can help find a correlation between geno- type and phenotype in the future.

The frameshift variant “p.(Asn50MetfsTer26)” was not previously reported in *CDKL5*. So far, more than 265 variants in this gene have been reported.[60](#_bookmark45) As far as we know, we judge that our novel frameshift variation corre- sponds to the second frameshift *CDKL5* variant recorded in Tunisian patients after the “p.(Glu930GlyfsTer9)”.[44](#_bookmark38) In this population, six other variants are identified at the level of this gene which all correspond to missense vari- ants.[45](#_bookmark39) The identified variant in this study, as well as all previously reported variants in *CDKL5* in Tunisia, were

associated with IESS. Our data offer soundproof and more comprehensive information to confirm that *CDKL5* is a potential gene for IESS in our population.

The nonsense variant “p.(Arg1206Ter)” in *SZT2* was detected in a homozygous state in our patient from a con- sanguineous family. This variant has not been previously reported in the homozygous state in control databases. Our study was conducted on the first set of siblings with ho- mozygosity for the “p.(Arg1206Ter)” variant in *SZT2*. This result further indicates biallelic variants in this gene as a cause of DEE. According to the literature, only 24 patients carrying variants in *SZT2* have been reported with a wide phenotypic spectrum from mild intellectual disability with- out epilepsy to DEE.[61](#_bookmark46) Other relevant characteristics refer to such macrocephaly and radiological findings as neuro- nal migration disorders and corpus callosum abnormali- ties, which might refer to the hyperactivation of mTORC1 signaling.[61](#_bookmark46) Though the genotype-phenotype correlation in *SZT2* variants remains ambiguous, frameshift variants proved to result in hyperactivation of mTORC1 signal- ing.[62–64](#_bookmark47) The identification of a homozygous variation in a consanguineous family reveals the role of inbreeding in the onset of autosomal recessive DEE. A study conducted on a non-consanguineous Caucasian population revealed a high contribution of recessive inheritance in DEE but with compound heterozygous variants.[65](#_bookmark48) In our study, de- spite the consanguinity, we have only one patient with au- tosomal recessive inheritance. It is possible that variations which are not covered with our panel or copy number vari- ations are responsible for an autosomal recessive DEE in negative patients.

The detected in-frame variant in *GNAO1* “p.(Leu- 39del)” was classified as “likely pathogenic” referring to ACMG and was not found in control population da- tabases. Furthermore, leucine at position 39 was highly conserved between species with a PhyloP100 conservation score equal to 7.53. So far, 50 *GNAO1* variants have been found in patients with movement disorders and epilep- tic encephalopathy, where three variants are in-frame: “p.(Ala301del),” “p.(Ala338del),” and “p.(Ile344del),”[66](#_bookmark49) These three reported patients did not present epilepsy or presented a single seizure, and they all share problems in motor development, with normal EEG findings, with or without intellectual disability.[67,68](#_bookmark50) Our patient SEED.0020 presents similar clinical findings. In fact, epileptic seizures appear notably at the age of 1 month. Still, these seizures disappear completely at the age of 2 years and 9 months and the patient presents intellectual deficiency, problems in motor development as well as normal EEG findings at this age. A second variant “p.(Leu23Pro)” was reported previously in the same domain containing the p.Leu39del variant (N-terminus domain, prior to the first G-motif), in a patient with the same phenotype[69](#_bookmark51) (Table [S3](#_bookmark65)).

Furthermore, the “p.(Asp479His)” in *PCDH19* is a newly discovered variant that proves to be severe. This novel variant is located in the fifth cadherin domain of the PCDH19 protein. This domain contains six reported mis- sense variants involved in epilepsy[19,70–73](#_bookmark22) (Table [S3](#_bookmark65)). An al- ternative variant at the same position “p.(Asp479Asn)” was classified as “likely pathogenic” by ClinVar. The affected mother possessed also the variant in the heterozygous state. The presence of variants with maternal inheritance in *PCDH19* was described four times in state of art works.[74–76](#_bookmark52) Moreover, it was emphasized that variants in *PCDH19* are associated with seizures occurring in clusters, provoked by fever with cognitive impairment.[77](#_bookmark53) Our patient SEED.0074 presents also this clinical phenotype (Table [1](#_bookmark3)).

A previously reported variant p.(Arg504Trp) was de- tected in *GRIN2A*[34,35](#_bookmark33) in a patient presenting DEE with Spike-Wave-Activation in Sleep (SWAS). The same variant was reported in patients with similar phenotypes present- ing Landau-Kleffner syndrome (LKS) or epileptic enceph- alopathy with continuous spike-and-wave during sleep (CSWS) and attention deficit and hyperactivity disorder (ADHD). However, this is the first report of *GRIN2A* vari- ants in the Tunisian population.

According to the varsome database ([https://varsome.](https://varsome.com/) [com](https://varsome.com/)), the p.(Arg145Cys) missense variant in *MECP2* is re- ported more than 140 times in the literature. This variant is present in a hot spot region[78](#_bookmark54) and classified as Pathogenic by ClinVar and UniProt. Additionally, there are 4 alterna- tive variants in the same position ((p.(Arg145Lys); p.(Ar- g145Leu); p.(Arg145His); and p.(Arg145Gly)), classified as “Pathogenic” by ClinVar and implicated in Rett syndrome or intellectual developmental disorders. In Tunisia, other vari- ants were reported in *MECP2* in patients with Rett syndrome, but this is the first report of p.(Arg145Cys) variant.[79–87](#_bookmark55)

Additionally, we identified the novel frameshift variant p.(Arg716GlyfsTer10) in *SYNGAP1*. Approximately 200 cases were reported worldwide, and most of these patients are from Europe.[88](#_bookmark56) The phenotype detected in our patient is in good agreement with previous studies showing that vari- ants in *SYNGAP1* are responsible for a clinical syndrome characterized by intellectual disability and epilepsy.[89](#_bookmark57) This is the first report of a variant in this gene on the African continent. Therefore, the *SYNGAP1* gene should be ana- lyzed in the future in patients with this syndrome.

## | Significance of genetic studies in different populations

**for the assessment of population-based disease-causing epilepsy genes**

Since 2014, several studies in various populations in America and Europe have used targeted NGS-based

**TABLE 3** Studies using gene panels for the diagnosis of DEE.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Population's origin** | **Number of tested individuals** | **Number of screened genes** | **Diagnosis rate (%) (pathogenic or likely pathogenic variants)** | **Most frequently affected genes** | **Reference** |
| United States | 13 | 38–53 | 46.2 | *PCDH19*, *SCN1A*, *SLC2A1*, *SPTAN1*, *SLC9A6*, *EFHC* | [[91](#_bookmark59)] |
| Denmark, Estonia, the UK, Argentina, and Pakistan | 46 | 216 | 23 | *SCN1A*, *CDKL5*, *GABRA1*, *GABRB3*, *KCNQ2*, *SCN2A*, *SCN8A*, *SLC2A1*, *STXBP1* | [[13](#_bookmark16)] |
| United States | 339 | 110 | 18 | *TSC2*, *SCN1A*, *KCNQ2*, *CDKL5*, *SCN2A*, *SCN8A*, *SCN1B*, | [[92](#_bookmark60)] |

*STXBP1*, *TPP1*, *PCDH19*, *CACNA1A*, *GABRA1*, *GRIN2A*, *SLC2A1*

South Korea

74

172

37.8

*SXTBP1*, *CDKL5*, *KCNQ2*, *SCN1A*, *SYNGAP1*, *GNAO1*, *KCNT1*

[[93](#_bookmark61)]

Korea 116 40 34.5 *SCN1A*, *PRRT2*, *ARX*, *SCN2A*, *KCNQ2*, *PCDH19*, *STXBP1*,

*DEPDC5*, *SCN8A*

[[94](#_bookmark62)]

Denmark

200

45–580

23

*SCN1A*, *KCNT1*, *STXBP1*, *SLC2A1*, *ATP6A1V*, *HNRNPU*, *MEF2C*, *IRF2BPL*

[[14](#_bookmark17)]

Germany 91 5–434 18 *SCN1A*, *TSC1*, *SCN8A*, *SYNGAP1*, *CPT2*, *KCNB1*, *PCDH19*,

*KCNQ2*, *CHD2*, *CACNA1A*, *STXBP1*

[[95](#_bookmark63)]

**|**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Turkey | 80 | 110 | 36.25 | *TSC2*, *TSC1*, *KCNQ2*, *AMT*, *CACNA1H*, *KCNT1*, *SCN1A*, *GRIN2A*, *CNTNAP2*, *GLDC*, *MECP2*, *ASAH1* | [[16](#_bookmark19)] |
| South Africa | 41 | 308 | 43.9 | *SCN1A*, *KANSL1*, *KCNQ2*, *CDKL5, IQSEC2*, *MC1A*, *STXBP1* | [[6](#_bookmark9)] |
| Tunisia | 40 | 116 | 30 | *SCN1A*, *CHD2*, *CDKL5*, *SZT2*, *KCNT1*, *GNAO1*, *PCDH19*, | Present study |
| *GRIN2A*, *MECP2*, *SYNGAP1* | | | | | |

epilepsy gene panels to identify the responsible genes for DEE. These panels may include between 5 and 580 genes, with a diagnostic yield ranging between 18% and 46% (Table [3](#_bookmark5)). In Africa, only one study has recently been published which was concerned with the South African population.[6](#_bookmark9) However, in North Africa (Tunisia, Egypt, Libya, Algeria, Morocco), no study on this issue has been undertaken before. In the Arabic countries, only one study was published in the Saudi Arabia region using WES/WGS sequencing rather than panels.[90](#_bookmark58) From this perspective, this is the first study that uses a personalized panel of high-throughput sequencing in the Tunisian as well as the North African region with a diagnosis rate of 30% (Table [3](#_bookmark5)). In our study, we specified Pathogenic or likely pathogenic variants in 10 genes (*SCN1A*, *CHD2*, *CDKL5*, *SZT2*, *KCNT1*, *GNAO1*, *PCDH19*, *MECP2*,

*GRIN2A*, and *SYNGAP1*) involved in DEE, and *SCN1A* seems to be the most frequently affected gene in Tunisia (Table [2](#_bookmark4)).

# | CONCLUSIONS

This study allows a deeper and better insight into the un- derlying causative genes and variants of DEE in Tunisian children. The 30% diagnostic yield goes in good conform- ity with previously reported international pediatric co- horts. Gene-directed therapies will be further enhanced in the future in a way that the management of all patients with DEE would be highly facilitated.

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### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are avail- able in the article. If additional data were required, they might be requested to the corresponding author.

### ETHICS STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. This study was approved by the Ministry of Higher Education and Scientific Research of Tunisia. All procedures performed in studies involving human participants were in accord- ance with the guidelines of the Regional Committee of the Protection of Persons, Sfax, Tunisia (CPP SUD reference number 28/2019).

### REFERENCES

1. Epilepsy: a public health imperative. Summary. Geneva: World Health Organization; 2019. (WHO/MSD/MER/19.2). Licence: CC BY-NC-SA 3.0 IGO.
2. Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, et al. ILAE classification of the epilepsies: posi- tion paper of the ILAE Commission for Classification and Terminology. Epilepsia. 2017;58:512–21.
3. Fisher RS, Cross JH, French JA, Higurashi N, Hirsch E, Jansen FE, et al. Operational classification of seizure types by the in- ternational league against epilepsy: position paper of the ILAE Commission for Classification and Terminology. Epilepsia. 2017;58:522–30.
4. Zuberi SM, Wirrell E, Yozawitz E, Wilmshurst JM, Specchio N, Riney K, et al. ILAE classification and definition of epi- lepsy syndromes with onset in neonates and infants: position statement by the ILAE task force on nosology and definitions. Epilepsia. 2022;63:1349–97.
5. Specchio N, Curatolo P. Developmental and epileptic encephalop- athies: What we do and do not know. Brain. 2021;144:32–43.
6. Essajee F, Urban M, Smit L, Wilmshurst JM, Solomons R, van Toorn R, et al. Utility of genetic testing in children with developmental and epileptic encephalopathy (DEE) at a ter- tiary hospital in South Africa: a prospective study. Seizure. 2022;101:197–204.
7. Amadori E, Scala M, Cereda GS, Vari MS, Marchese F, Di Pisa V, et al. Targeted re-sequencing for early diagnosis of genetic causes of childhood epilepsy: the Italian experience from the ‘beyond epilepsy'project. Ital J Pediatr. 2020;46:1–9.
8. Scala M, Bianchi A, Bisulli F, Coppola A, Elia M, Trivisano M, et al. Advances in genetic testing and optimization of clinical management in children and adults with epilepsy. Expert Rev Neurother. 2020;20:251–69.
9. Desvignes J-P, Bartoli M, Delague V, Krahn M, Miltgen M, Béroud C, et al. VarAFT: a variant annotation and filtration sys- tem for human next generation sequencing data. Nucleic Acids Res. 2018;46:W545–53.
10. 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. Genet Med. 2015;17:405–23.
11. Choi Y, Chan AP. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics. 2015;31:2745–7.
12. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods. 2014;11:361–2.
13. Moller RS, Larsen LH, Johannesen KM, Talvik I, Talvik T, Vaher U, et al. Gene panel testing in epileptic encephalopathies and familial epilepsies. Mol Syndromol. 2016;7:210–9.
14. Johannesen KM, Nikanorova N, Marjanovic D, Pavbro A, Larsen LHG, Rubboli G, et al. Utility of genetic testing for therapeutic decision-making in adults with epilepsy. Epilepsia. 2020;61:1234–9.
15. Specchio N, Pietrafusa N, Doccini V, Trivisano M, Darra F, Ragona F, et al. Efficacy and safety of Fenfluramine hydro- chloride for the treatment of seizures in Dravet syndrome: a real-world study. Epilepsia. 2020;61:2405–14.
16. Atli EI, Atli E, Yalcintepe S, Demir S, Kalkan R, Eker D, et al. Customised targeted massively parallel sequencing enables more precise diagnosis of patients with epilepsy. Intern Med J. 2022;52:1174–84.
17. Kearney JA, Wiste AK, Stephani U, Trudeau MM, Siegel A, RamachandranNair R, et al. Recurrent de novo mutations of SCN1A in severe myoclonic epilepsy of infancy. Pediatr Neurol. 2006;34:116–20.
18. Mancardi MM, Striano P, Gennaro E, Madia F, Paravidino R, Scapolan S, et al. Familial occurrence of febrile seizures and ep- ilepsy in severe myoclonic epilepsy of infancy (SMEI) patients with SCN1A mutations. Epilepsia. 2006;47:1629–35.
19. Depienne C, Bouteiller D, Keren B, Cheuret E, Poirier K, Trouillard O, et al. Sporadic infantile epileptic enceph- alopathy caused by mutations in PCDH19 resembles Dravet syndrome but mainly affects females. PLoS Genet. 2009;5:e1000381.
20. Sun H, Zhang Y, Liu X, Ma X, Wu H, Xu K, et al. Mutation anal- ysis of the SCN1A gene in severe myoclonic epilepsy of infancy. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2009;26:121–7.
21. Rodda JM, Scheffer IE, McMahon JM, Berkovic SF, Graham HK. Progressive gait deterioration in adolescents with Dravet syndrome. Arch Neurol. 2012;69:873–8.
22. Xu X, Yang X, Wu Q, Liu A, Yang X, Ye AY, et al. Amplicon resequencing identified parental mosaicism for approximately 10% of "de novo" SCN1A mutations in children with Dravet syn- drome. Hum Mutat. 2015;36:861–72.
23. He N, Lin ZJ, Wang J, Wei F, Meng H, Liu XR, et al. Evaluating the pathogenic potential of genes with de novo variants in epi- leptic encephalopathies. Genet Med. 2019;21:17–27.
24. Dilena R, DiFrancesco JC, Soldovieri MV, Giacobbe A, Ambrosino P, Mosca I, et al. Early treatment with quinidine in 2 patients with epilepsy of infancy with migrating focal seizures (EIMFS) due to gain-of-function KCNT1 mutations: functional studies Clinical responses, critical issues for personalized ther- apy. Neurotherapeutics. 2018;15:1112–26.
25. Rubboli G, Plazzi G, Picard F, Nobili L, Hirsch E, Chelly J, et al. Mild malformations of cortical development in sleep-re- lated hypermotor epilepsy due to KCNT1 mutations. Ann Clin Translat Neurol. 2019;6:386–91.
26. Fitzgerald MP, Fiannacca M, Smith DM, Gertler TS, Gunning B, Syrbe S, et al. Treatment responsiveness in KCNT1-related epilepsy. Neurotherapeutics. 2019;16:848–57.
27. Borlot F, Abushama A, Morrison-Levy N, Jain P, Puthenveettil Vinayan K, Abukhalid M, et al. KCNT1-related epilepsy: An in- ternational multicenter cohort of 27 pediatric cases. Epilepsia. 2020;61:679–92.
28. Moller RS, Heron SE, Larsen LH, Lim CX, Ricos MG, Bayly MA, et al. Mutations in KCNT1 cause a spectrum of focal epilepsies. Epilepsia. 2015;56:e114–20.
29. Costain G, Cordeiro D, Matviychuk D, Mercimek-Andrews S. Clinical application of targeted next-generation sequencing panels and whole exome sequencing in childhood epilepsy. Neuroscience. 2019;418:291–310.
30. Myers CT, Hollingsworth G, Muir AM, Schneider AL, Thuesmunn Z, Knupp A, et al. Parental mosaicism in "De novo" epileptic encephalopathies. N Engl J Med. 2018;378:1646–8.
31. Burgess R, Wang S, McTague A, Boysen KE, Yang X, Zeng Q, et al. The genetic landscape of epilepsy of infancy with migrat- ing focal seizures. Ann Neurol. 2019;86:821–31.
32. McTague A, Nair U, Malhotra S, Meyer E, Trump N, Gazina EV, et al. Clinical and molecular characterization of KCNT1-related severe early-onset epilepsy. Neurology. 2018;90:e55–66.
33. Mullen SA, Carney PW, Roten A, Ching M, Lightfoot PA, Churilov L, et al. Precision therapy for epilepsy due to KCNT1 mutations: a randomized trial of oral quinidine. Neurology. 2018;90:e67–72.
34. Lesca G, Rudolf G, Bruneau N, Lozovaya N, Labalme A, Boutry-Kryza N, et al. GRIN2A mutations in acquired epilep- tic aphasia and related childhood focal epilepsies and enceph- alopathies with speech and language dysfunction. Nat Genet. 2013;45:1061–6.
35. Li X, Xie LL, Han W, Hong SQ, Ma JN, Wang J, et al. Clinical forms and GRIN2A genotype of severe end of epileptic-aphasia Spectrum disorder. Front Pediatr. 2020;8:574803.
36. Shi XY, Tomonoh Y, Wang WZ, Ishii A, Higurashi N, Kurahashi H, et al. Efficacy of antiepileptic drugs for the treatment of Dravet syndrome with different genotypes. Brain Dev. 2016;38:40–6.
37. Wirrell EC, Laux L, Donner E, Jette N, Knupp K, Meskis MA, et al. Optimizing the diagnosis and Management of Dravet Syndrome: recommendations from a north American consen- sus panel. Pediatr Neurol. 2017 Mar;68:18.e3–34.e3.
38. Chiron C, Marchand MC, Tran A, Rey E, d'Athis P, Vincent J, et al. Stiripentol in severe myoclonic epilepsy in infancy: a ran- domised placebo-controlled syndrome-dedicated trial. STICLO Study Group Lancet. 2000;356:1638–42.
39. Nabbout R, Mistry A, Zuberi S, Villeneuve N, Gil-Nagel A, Sanchez-Carpintero R, et al. Fenfluramine for treatment-re- sistant seizures in patients with Dravet syndrome receiving

Stiripentol-inclusive regimens: A randomized clinical trial. JAMA Neurol. 2020;77:300–8.

1. Ko A, Jung DE, Kim SH, Kang HC, Lee JS, Lee ST, et al. The efficacy of ketogenic diet for specific genetic mutation in developmental and epileptic encephalopathy. Front Neurol. 2018;9:530.
2. Devinsky O, Cross JH, Wright S. Trial of Cannabidiol for drug-resistant seizures in the Dravet syndrome. The New England Journal of Medicine. 2017;377:699–700.
3. Sadleir LG, Kolc KL, King C, Mefford HC, Dale RC, Gecz J, et al. Levetiracetam efficacy in PCDH19 girls clustering epilepsy European journal of paediatric neurology. Eur Paediatr Neurol. 2020;24:142–7.
4. Knight EMP, Amin S, Bahi-Buisson N, Benke TA, Cross JH, Demarest ST, et al. Safety and efficacy of ganaxolone in patients with CDKL5 deficiency disorder: results from the double-blind phase of a randomised, placebo-controlled, phase 3 trial. Lancet Neurol. 2022;21:417–27.
5. Jdila MB, Triki C, Rhouma BB, Jomaa RB, Issa AB, Ammar- Keskes L, et al. A novel C-terminal truncated mutation in hCDKL5 protein causing a severe West syndrome: comparison with previous truncated mutations and genotype/phenotype correlation. Int J Dev Neurosci. 2019;72:22–30.
6. Jdila MB, Issa AB, Khabou B, Rhouma BB, Kamoun F, Ammar- Keskes L, et al. Novel mutations in the CDKL5 gene in complex genotypes associated with West syndrome with variable pheno- type: first description of somatic mosaic state. Clin Chim Acta. 2017;473:51–9.
7. Jdila MB, Mignon-Ravix C, Ncir SB, Kammoun F, Fakhfakh F, Villard L, et al. A large consanguineous family with a homozy- gous metabotropic glutamate receptor 7 (mGlu7) variant and de- velopmental epileptic encephalopathy: effect on protein structure and ligand affinity. Orphanet J Rare Dis. 2021;16:317.
8. Kamoun Feki F, Fendri Kriaa N, Kolsi D, Rabai A, Fakhfakh F, Charfi TC. Clinical and genetic aspect of 30 tunisian families with febrile seizures. Tunis Med. 2019;97:525–32.
9. Ben Mahmoud A, Ben Mansour R, Driss F, Baklouti-Gargouri S, Siala O, Mkaouar-Rebai E, et al. Evaluation of the effect of c.2946+1G>T mutation on splicing in the SCN1A gene. Comput Biol Chem. 2015;54:44–8.
10. Fendri-Kriaa N, Boujilbene S, Kammoun F, Mkaouar-Rebai E, Ben Mahmoud A, Hsairi I, et al. A putative disease-associ- ated haplotype within the SCN1A gene in Dravet syndrome. Biochem Biophys Res Commun. 2011;408:654–7.
11. Fendri-Kriaa N, Kammoun F, Rebai A, Kolsi D, Hadj Salem I, Fakhfakh F, et al. Genetic screening of two Tunisian fami- lies with generalized epilepsy with febrile seizures plus. Eur J Neurol. 2009;16:697–704.
12. Fendri-Kriaa N, Kammoun F, Salem IH, Kifagi C, Mkaouar- Rebai E, Hsairi I, et al. New mutation c374C>T and a putative disease-associated haplotype within SCN1B gene in Tunisian families with febrile seizures. Eur J Neurol. 2011;18:695–702.
13. Tlili A, Hamida Hentati N, Chaabane R, Gargouri A, Fakhfakh

F. Pyridoxine-dependent epilepsy in Tunisia is caused by a founder missense mutation of the ALDH7A1 gene. Gene. 2013;518:242–5.

1. Tlili A, Hamida Hentati N, Gargouri A, Fakhfakh F. Identification of a novel missense mutation in the ALDH7A1 gene in two unrelated Tunisian families with pyridoxine-de- pendent epilepsy. Mol Biol Rep. 2013;40:487–90.
2. Depienne C, Trouillard O, Saint-Martin C, Gourfinkel-An I, Bouteiller D, Carpentier W, et al. Spectrum of SCN1A gene mutations associated with Dravet syndrome: analysis of 333 pa- tients. J Med Genet. 2009;46:183–91.
3. Li W, Schneider AL, Scheffer IE. Defining Dravet syndrome: An essential pre-requisite for precision medicine trials. Epilepsia. 2021;62:2205–17.
4. Hildebrand MS, Myers CT, Carvill GL, Regan BM, Damiano JA, Mullen SA, et al. A targeted resequencing gene panel for focal epilepsy. Neurology. 2016;86:1605–12.
5. Rehm HL, Berg JS, Brooks LD, Bustamante CD, Evans JP, Landrum MJ, et al. ClinGen–the clinical genome resource. N Engl J Med. 2015;372:2235–42.
6. De Maria B, Balestrini S, Mei D, Melani F, Pellacani S, Pisano T, et al. Expanding the genetic and phenotypic spectrum of CHD2- related disease: from early neurodevelopmental disorders to adult-onset epilepsy. Am J Med Genet A. 2022;188:522–33.
7. Hanly C, Shah H, Au PYB, Murias K. Description of neurodevelop- mental phenotypes associated with 10 genetic neurodevelopmen- tal disorders: a scoping review. Clin Genet. 2021;99:335–46.
8. Jakimiec M, Paprocka J, Smigiel R. CDKL5 deficiency disorder-A complex epileptic encephalopathy. Brain Sci. 2020;10:107.
9. Trivisano M, Rivera M, Terracciano A, Ciolfi A, Napolitano A, Pepi C, et al. Developmental and epileptic encephalopathy due to SZT2 genomic variants: emerging features of a syndromic condition. Epilepsy Behav. 2020;108:107097.
10. Nakamura Y, Kato K, Tsuchida N, Matsumoto N, Takahashi Y, Saitoh S. Constitutive activation of mTORC1 signaling in- duced by biallelic loss-of-function mutations in SZT2 under- lies a discernible neurodevelopmental disease. PLoS ONE. 2019;14:e0221482.
11. Peng M, Yin N, Li MO. SZT2 dictates GATOR control of mTORC1 signalling. Nature. 2017;543:433–7.
12. Wolfson RL, Chantranupong L, Wyant GA, Gu X, Orozco JM, Shen K, et al. KICSTOR recruits GATOR1 to the lysosome and is neces- sary for nutrients to regulate mTORC1. Nature. 2017;543:438–42.
13. Papuc SM, Abela L, Steindl K, Begemann A, Simmons TL, Schmitt B, et al. The role of recessive inheritance in early-onset epileptic encephalopathies: a combined whole-exome sequencing and copy number study. Eur J Hum Genet. 2019;27:408–21.
14. Ling W, Huang D, Yang F, Yang Z, Liu M, Zhu Q, et al. Treating GNAO1 mutation-related severe movement disorders with ox- carbazepine: a case report. Transl Pediatr. 2022;11:1577–87.
15. Kim SY, Shim Y, Ko YJ, Park S, Jang SS, Lim BC, et al. Spectrum of movement disorders in GNAO1 encephalopathy: in-depth phenotyping and case-by-case analysis. Orphanet J Rare Dis. 2020;15:343.
16. Kelly M, Park M, Mihalek I, Rochtus A, Gramm M, Perez- Palma E, et al. Spectrum of neurodevelopmental disease associ- ated with the GNAO1 guanosine triphosphate-binding region. Epilepsia. 2019 Mar;60:406–18.
17. Wirth T, Garone G, Kurian MA, Piton A, Millan F, Telegrafi A, et al. Highlighting the dystonic phenotype related to GNAO1. Mov Disord. 2022;37:1547–54.
18. Specchio N, Marini C, Terracciano A, Mei D, Trivisano M, Sicca F, et al. Spectrum of phenotypes in female patients with epilepsy due to protocadherin 19 mutations. Epilepsia. 2011;52:1251–7.
19. Wang J, Zhang J, Yang Y, Gao K, Wu Y, Zhang Y, et al. Efficacy of ketogenic diet for infantile spasms in Chinese patients with or without monogenic etiology. Front Pediatr. 2022;10:842666.
20. Dibbens LM, Tarpey PS, Hynes K, Bayly MA, Scheffer IE, Smith R, et al. X-linked protocadherin 19 mutations cause fe- male-limited epilepsy and cognitive impairment. Nat Genet. 2008;40:776–81.
21. Carvill GL, Heavin SB, Yendle SC, McMahon JM, O'Roak BJ, Cook J, et al. Targeted resequencing in epileptic encephalopa- thies identifies de novo mutations in CHD2 and SYNGAP1. Nat Genet. 2013;45:825–30.
22. Depienne C, Trouillard O, Bouteiller D, Gourfinkel-An I, Poirier K, Rivier F, et al. Mutations and deletions in PCDH19 account for various familial or isolated epilepsies in females. Hum Mutat. 2011;32:E1959–75.
23. Dibbens LM, Kneen R, Bayly MA, Heron SE, Arsov T, Damiano JA, et al. Recurrence risk of epilepsy and mental retardation in females due to parental mosaicism of PCDH19 mutations. Neurology. 2011;76:1514–9.
24. Dimova PS, Kirov A, Todorova A, Todorov T, Mitev V. A novel PCDH19 mutation inherited from an unaffected mother. Pediatr Neurol. 2012;46:397–400.
25. Yang L, Liu J, Su Q, Li Y, Yang X, Xu L, et al. Novel and de novo mutation of PCDH19 in girls clustering epilepsy. Brain Behav. 2019;9:e01455.
26. Laccone F, Huppke P, Hanefeld F, Meins M. Mutation spectrum in patients with Rett syndrome in the German population: evi- dence of hot spot regions. Hum Mutat. 2001;17:183–90.
27. Fendri-Kriaa N, Abdelkafi Z, Rebeh IB, Kamoun F, Triki C, Fakhfakh F. A novel MECP2 gene mutation in a Tunisian patient with Rett syndrome. Genet Test Mol Biomarkers. 2009;13:109–13.
28. Fendri-Kriaa N, Hsairi I, Kifagi C, Ellouze E, Mkaouar-Rebai E, Triki C, et al. A case of a Tunisian Rett patient with a novel double-mutation of the MECP2 gene. Biochem Biophys Res Commun. 2011;409:270–4.
29. Fendri-Kriaa N, Mkaouar-Rebai E, Moalla D, Belguith N, Louhichi N, Zemni R, et al. Mutational analysis of the MECP2 gene in Tunisian patients with Rett syndrome: a novel double mutation. J Child Neurol. 2010;25:1042–6.
30. Fendri-Kriaa N, Rouissi A, Ghorbel R, Mkaouar-Rebai E, Belguith N, Gouider-Khouja N, et al. Novel double deletions in the MECP2 gene in Tunisian Rett patient. Gene. 2012;502:163–7.
31. Fendri-Kriaa N, Rouissi A, Ghorbel R, Mkaouar-Rebai E, Belguith N, Gouider-Khouja N, et al. Novel mutations in the C-terminal region of the MECP2 gene in Tunisian Rett syn- drome patients. J Child Neurol. 2012;27:564–8.
32. Ghorbel R, Ghorbel R, Rouissi A, Fendri-Kriaa N, Ben Salah G, Belguith N, et al. First report of an unusual novel double mu- tation affecting the transcription repression domain of MeCP2 and causing a severe phenotype of Rett syndrome: molecular analyses and computational investigation. Biochem Biophys Res Commun. 2018;497:93–101.
33. Kharrat M, Hsairi I, Doukali H, Fendri-Kriaa N, Kammoun H, Ammar-Keskes L, et al. Phenotypic variability in two infants sharing the same MECP2 mutation: evidence of chromosomal rearrangements and high sister-chromatid exchange levels in Rett syndrome. Acta Neurol Belg. 2017;117:251–8.
34. Kharrat M, Kamoun Y, Kamoun F, Ellouze E, Maalej M, Fendri- Kriaa N, et al. Clinical, molecular, and computational analysis

in patients with a novel double mutation and a new synony- mous variant in MeCP2: report of the first missense mutation within the AT-hook1 cluster in Rett syndrome. J Child Neurol. 2017;32:694–703.

1. Kharrat M, Hsairi I, Fendri-Kriaa N, Kenoun H, Othmen HB, Ben Mahmoud A, et al. A novel mutation p.A59P in N-terminal domain of methyl-CpG-binding protein 2 confers phenotypic variability in 3 cases of Tunisian Rett patients: clin- ical evaluations and in Silico investigations. J Child Neurol. 2015;30:1715–21.
2. Zhang H, Yang L, Duan J, Zeng Q, Chen L, Fang Y, et al. Phenotypes in children with SYNGAP1 encephalopathy in China. Front Neurosci. 2021;15:761473.
3. Agarwal M, Johnston MV, Stafstrom CE. SYNGAP1 mutations: clinical, genetic, and pathophysiological features. Int J Dev Neurosci. 2019;78:65–76.
4. Alsubaie L, Aloraini T, Amoudi M, Swaid A, Eyiad W, Al Mutairi F, et al. Genomic testing and counseling: the contribu- tion of next-generation sequencing to epilepsy genetics. Ann Hum Genet. 2020;84:431–6.
5. Ream MA, Mikati MA. Clinical utility of genetic testing in pe- diatric drug-resistant epilepsy: a pilot study. Epilepsy Behav. 2014;37:241–8.
6. Butler KM, da Silva C, Alexander JJ, Hegde M, Escayg A. Diagnostic yield from 339 epilepsy patients screened on a clini- cal gene panel. Pediatr Neurol. 2017;77:61–6.
7. Rim JH, Kim SH, Hwang IS, Kwon SS, Kim J, Kim HW, et al. Efficient strategy for the molecular diagnosis of intractable ear- ly-onset epilepsy using targeted gene sequencing. BMC Med Genom. 2018;11:1–10.
8. Lee J, Lee C, Ki CS, Lee J. Determining the best candidates for next-generation sequencing-based gene panel for evaluation of early-onset epilepsy. Mol Genet Genom Med. 2020;8:e1376.
9. Hoelz H, Herdl C, Gerstl L, Tacke M, Vill K, von Stuelpnagel C, et al. Impact on clinical decision making of next-generation sequencing in pediatric epilepsy in a tertiary epilepsy referral center. Clin EEG Neurosci. 2020;51:61–9.

### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.