Received: 11 October 2024 | Revised: 8 April 2025 | Accepted: 29 April 2025

DOI: 10.1002/epi4.70057

Epilepsia Open® Open Access

ORIGINAL ARTICLE

Genetic etiologies with a large NGS panel in a monocentric cohort of 1000 patients with pediatric onset epilepsies

Giulia Barcia[1,2,3] | **Nicole Chemaly**[3,4] | **Stéphanie Gobin-Limballe**[1] | **Emma Losito**[3,4,5] | **Mélodie Aubart**[4] | **Eugénie Sarda**[4] | **Zahra Assouline**[1,2] | **Pauline Plante-Bordeneuve**[1,2] | **Marie Hully**[4] | **Remi Barrois**[3,4,5] | **Christine Barnerias**[4] | **Doxa Sareidaki**[3,4] | **Delphine Coste Zeitoun**[3,5] | **Monika Eisermann**[3,5] | **Cécile Fourrage**[1] | **Sylvain Hanein**[2] | **Marlène Rio**[1] | **Nathalie Boddaert**[6] | **Isabelle Desguerre**[4] | **Anna Kaminska**[3,5] | **Julie Steffann**[1,2] | **Rima Nabbout**[2,3,4]

[1]Service de Médecine Génomique, Necker Enfants-Malades Hospital, APHP, Université de Paris, Paris, France

[2]INSERM U1163, Imagine Institute for Rare Diseases, Université de Paris, Paris, France

[3]Necker Enfants-Malades Hospital, Membre of EPICARE, Reference Center for Rare Epilepsies, Paris, France

[4]Department of Pediatric Neurology, Necker Enfants-Malades Hospital Université de Paris Cité, Paris, France

[5]Department of Clinical Neurophysiology, Necker Enfants-Malades Hospital, APHP, Paris, France

[6]Department of Pediatric Radiology, Necker Enfants-Malades Hospital, APHP, Paris, France

Correspondence
Giulia Barcia, Service de Médecine Génomique, Necker Enfants-Malades Hospital, APHP, Université de Paris, Paris, France, 149 rue de Sèvres, Paris 75015, France.
Email: giulia.barcia@aphp.fr

Abstract

Objective: Genetic testing is now included in the diagnostic assessment of childhood onset epilepsies. We evaluated the yield of a targeted next generation sequencing (TNGS) panel dedicated to pediatric epilepsies.

Methods: We tested by TNGS panel 1000 consecutive patients presenting with childhood onset epilepsies and including mainly patients with early onset epilepsies (under 2 years, 61%).

Results: Causal variants were identified in 31% of patients, spanning 78 different genes. Patients with benign familial neonatal/infantile epilepsy (BFN/IS) exhibited the highest rate of positive findings (82%). Developmental and epileptic encephalopathies (DEEs) had a global diagnostic yield of 37%, with epilepsy of infancy with migrating focal seizures (EIMFSI) and Dravet syndrome (DS) presenting the highest yield in this group (78%) and early infantile DEE (EIDEE) laying next with a yield of 43%. The lowest rates of genetic diagnosis were observed in infantile epileptic spasms syndrome (IESS, 17%), epilepsy with myoclonic-atonic seizures (EMAtS, 19%), and DEE-SWAS (14%). Patients with GEFS+ had a yield of 16%. Among patients with developmental encephalopathies and refractory seizures with onset after 2 years, TNGS yielded a 33% diagnostic rate. Atypical absences yielded 16%, focal epilepsy yielded 18%, and generalized epilepsies with refractory seizures yielded 13%. These groups exhibited a high genetic heterogeneity.

Significance: TNGS is an effective first-step genetic screening in patients with high diagnostic yields (BFN/IS, EIMFS, DS, EIDEE) and for epilepsy syndromes associated with one or a few major genes (BFN/IS, EIMFS, DS, GEFS+,

DEE-SWAS). Whole exome or genome sequencing (WES/WGS) should be considered as a second step in these groups with a probably relevant Mendelian inheritance. WES/WGS could be proposed as first-tier analysis in patients with IESS, EMAtS, generalized or focal epilepsies refractory to ASMs, and developmental encephalopathies with seizure onset after 2 years. However, the lower diagnostic yield obtained in these groups may suggest a complex inheritance.

Plain Language Summary: This study emphasizes the importance of accurately identifying different types of epilepsy and epilepsy syndromes to improve genetic testing strategies. We suggest that a targeted gene panel can be a good first step for some genetic conditions, such as benign familial neonatal/infantile epilepsy, Dravet syndrome, and epilepsy of infancy with migrating focal seizures.

KEYWORDS

developmental and epileptic encephalopathies, Dravet syndrome, epilepsy with migrating focal seizures of infancy, genetics, next generation sequencing

1 | INTRODUCTION

Over the past decades, the identification of genes involved in epileptic disorders has grown exponentially thanks to major advances in molecular technologies, notably the advent of next generation sequencing (NGS), coupled to careful and deep electro-clinical phenotyping. These achievements have transformed the diagnostic strategy dedicated to patients affected by childhood-onset epilepsy, leading to the integration of NGS into the diagnostic framework.[1,2]

A genetic diagnosis has significant positive implications for patients and their families. It can reduce unnecessary and often invasive investigations, facilitate therapy adjustment, and enable the use of targeted anti-seizure medications (ASMs) for specific etiologies. Furthermore, a genetic diagnosis provides patients and their families with insights into the underlying cause of their disease and allows genetic counseling with possible prenatal or preimplantation diagnosis.[2,3]

In 2015 we developed a custom targeted next generation sequencing (TNGS) strategy for the molecular diagnosis of pediatric epilepsy. We have prospectively assessed the efficacy of this approach across various electro-clinical syndromes. Our aim was to identify the electro-clinical phenotypes that would benefit most from TNGS as a first-line diagnostic tool. We integrated our results in the ILAE classification.[4]

Key points

- A precise phenotyping and the ILAE epilepsy syndrome classification are essential for guiding genetic diagnostic strategies, helping to identify the most appropriate testing methods.
- Targeted next-generation sequencing can serve as an initial alternative to whole exome/genome sequencing for certain monogenic syndromes with a high diagnostic yield.
- Whole exome sequencing or genome sequencing is recommended as a first-tier analysis for patients with specific conditions, including infantile epileptic spasms syndrome or epilepsy with myoclonic atonic seizures.

2 | METHODS

We included 1000 consecutive pediatric patients, followed in our reference center for rare epilepsies at Necker Enfants-Malades Hospital, between 2015 and 2023.

Electroclinical data were obtained for all patients. We detailed seizure history including age of onset, seizures' type, and response to antiseizure medications (ASMs). We evaluated the family history of seizure disorders, personal history, early psychomotor development, neurological examination, video-EEG recordings, and neuroimaging studies.

We included all patients with epilepsy onset before the age of 2 years except patients with evidence of brain anoxic-ischemic insult. Patients with confirmed malformation of cortical development and tuberous sclerosis were excluded as they are tested by dedicated panels.

For patients with seizure onset after the age of 2 years, we included self-limited focal epilepsies (SeLFE) evolving to a DEE/EE-SWAS spectrum, and patients with genetic generalized epilepsies (GGE) refractory to ASMs. Epilepsy syndrome diagnosis was made according to the ILAE criteria.[4]

Patients' inclusion in the panel and the results of the panel were discussed by a multidisciplinary team composed of pediatric neurologists, neurophysiologists, neuroradiologists, and molecular geneticists.

For patients included between 2015 and 2021, we used the first version of the panel targeting 151 genes. For patients included between 2022 and 2023, we used the second version of the panel targeting 249 genes. The panel design, the NGS technique, and data analysis are detailed in the Appendix S1 and S3. All patients or legal guardians gave written informed consent for genetic diagnosis procedures.

3 | RESULTS

3.1 | Population

We included 1000 consecutive patients (535 boys, 54%) with a mean age at seizure onset of 2 years (range: 0–13 years, median: 6 months). Of these, 164 (16%) had neonatal seizure onset, and 616 (62%) had seizures before 2 years of age. At the last follow-up, the mean age was 7.1 years (range: 1 month-36 years, median: 1.5 years). Consanguinity was reported in 6% of families, and 14% had a family history of seizures. Electroclinical diagnosis is detailed in Table 1.

TABLE 1 Electroclinical diagnosis and diagnostic yield of TNGS in this cohort.

Features of the cohort	No. of patients and age	Diagnostic TNGS yield
Patients	1000	31%
Mean age at epilepsy onset	2 years (range: 0–13 years, median: 6 month)	
Age at last follow-up	7.1 years (1 month–36 years, median: 1.5 years)	
Neonatal onset of seizures	164	
Diagnostic group	**No. of patients**	**Diagnostic TNGS yield**
Epilepsies with developmental and epileptic encephalopathy	463	37%
EIDEE	168	43%
IESS	132	17%
EMAtS	52	19%
DS	51	78%
EIMFS	23	78%
DEE-SWAS	21	14%
LGS	11	36%
HHE	5	40%
GEFS+	62	16%
Benign (familial) neonatal/infantile epilepsy	39	82%
Atypical absences	56	16%
Focal epilepsy refractory to ASMs	190	18%
Generalized epilepsy refractory to ASMs	52	13%
DE with epilepsy refractory to ASMs	138	33%

Abbreviations: ASMs, anti-seizure medication; DEE-SWAS, developmental and epileptic encephalopathy with spike–wave activity in sleep; DS, Dravet syndrome; EI-DEE, Early-infantile developmental and epileptic encephalopathy syndrome; EIMFS, epilepsy of infancy with migrating focal seizures; EMAtS, epilepsy with myoclonic-atonic seizures; GEFS+, generalized epilepsy with febrile seizures plus; HHE, hemiconvulsion hemiplegia epilepsy syndrome; IESS, infantile epileptic spasms syndrome; LGS, Lennox–Gastaut syndrome; m, months; TNGS, targeted next generation sequencing.

3.2 | Genetic results (NGS panel)

For the patients included in the first version of the panel, we obtained a mean depth of sequencing of 448× (range: 230–724×, median: 477×). For the patients included in the second version of the panel, we obtained a mean depth of sequencing of 585× (range: 331–732×, median: 540×).

Considering the whole cohort, a pathogenic or likely pathogenic variant (PV/LPV) was identified in 31% of patients (310/1000). Of the PV/LPVs, 49% (152/310) were novel. PV/LPV laid on 78 different genes (Appendix S2). A "top 10" of genes accounted for 18% (175/1000) of the genetic diagnosis, including *SCN1A* ($n = 48$), *KCNQ2* ($n = 28$), *SCN2A* ($n = 21$), *PRRT2* ($n = 17$), *SCN8A* ($n = 13$), *STXBP1* ($n = 11$), *KCNT1* ($n = 10$), *CDKL5* ($n = 8$), *SYNGAP1* ($n = 7$), *WWOX* ($n = 6$), and *CHD2* ($n = 6$, Appendix S2).

Autosomal dominant conditions were identified in 82% of patients (254/310). In this group, PV/LPVs were de novo in 56% (174/310), inherited from an affected heterozygous parent in 4% (12/310), inherited from a healthy heterozygous parent in 12% (36/310), and inherited from a parent carrying the variant in probable somatic mosaicism in 2% (5/310). In seven cases, a heterozygous PV/LPV variant was absent in one parent, but the segregation analysis could not be performed in the

second parent. In 20 cases, the segregation study could not be performed. Probable somatic mosaicism was detected in 3% of patients (10/310) ranging from 11% to 32% of the reads. A probable gonadal mosaicism was identified in one family (Appendix S2).

Autosomal recessive conditions were identified in 10% of patients (30/310). X-linked conditions were identified in 8% (26/310) including 21 de novo variants, 4 hemizygous variants inherited from the heterozygous unaffected mother, and 1 heterozygous variant inherited from the unaffected mother carrying the variant in somatic mosaicism (Appendix S2).

Six hundred sixty-one patients (661/1000) were included in the first version of the panel with a diagnostic yield of 30% (201/661). Three hundred thirty-nine patients (339/1000) were included in the second version of the panel with a diagnostic yield of 32% (109/339), (Figure 1A). For five patients (5/339) included in the second version of the panel, the pathogenic variant would have been missed by the first panel version, as they are located in genes that were added in the second version (*NUS1*, *KCNMA1*, *FGF12, UBA5*, and *SYNJ1*, Appendix S2).

In 2% of patients, we identified variants classified of unknown significance (VUS) based on several elements including a low frequency in the public databases (GnomAD), in silico prediction of pathogenicity, lack of precise genotype–phenotype correlation and the unavailability of the parental samples to perform the familial study (Appendix S2).

3.3 | Genetic results in relation to epilepsy syndromes

3.3.1 | Developmental and epileptic encephalopathies

PV/LPVs were found in 37% (172/463) of patients with DEE. In patients with EIDEE, a molecular diagnosis could be established in 43% of children (72/168) with mutations affecting 28 different genes. Mutations mainly affected *STXBP*1 ($n=9$), *SCN2A* ($n=8$), *KCNQ2* ($n=7$), *WWOX* ($n=6$), *DNM1* ($n=4$), *SCN8A* ($n=4$), and *CDKL5* ($n=4$). All EIDEE are de novo except for the autosomal recessive *WWOX*-EIDEE and EIDEE due to *RARS2*, *SYNJ1*, and *PNPO* LPV/PV (Table 1, Figures 1B–D, 2A, Appendix S2).

Considering patients with EIMFS, we identified a PV/LPV in 78% (18/23) with de novo variants in *KCNT1* ($n=9$), *SCN8A* ($n=2$), *KCNA2* ($n=1$), *KCNC1* ($n=1$), and *CDKL5* ($n=1$). In two patients, compound heterozygous variants were identified in *QARS* ($n=1$) and *ALDH7A1* ($n=1$). Two children had hemizygous variants in *PIGA* ($n=2$), de novo in one case and inherited from the unaffected heterozygous mother in the second (Table 1, Figures 1B–D, 2A, Appendix S2).

In patients with IESS, a de novo causal variant was identified in 17% (23/132) affecting 16 different genes: *CDKL5* ($n=3$), *IQSEC2* ($n=2$), *SCN2A* ($n=2$), *ALG13* ($n=2$), *GRIN2B* ($n=1$), *STXBP1* ($n=1$), *KCNQ2* ($n=1$), *GRIN2A* ($n=1$), *NEDD4L* ($n=1$), *GRIN1* ($n=1$), *SPTAN1* ($n=1$), *DEPDC5* ($n=1$), *MECP2* ($n=1$), *NIPA2* ($n=1$), *CACNA1E* ($n=1$), and *UBA5* ($n=1$). We identified a triplication including *GABRB3, NIPA2*, and *UBE3A* and confirmed by FISH of an iso-dicentric chromosome 15 in one patient. Finally, we detected a de novo 2q24.3 deletion encompassing *SCN2A* and *SCN1A* and a heterozygous *NIPA2* deletion confirmed by a CGH-array identifying the recurrent 15q11.2 BP1-BP2 deletion encompassing *NIPA2*. PV/LPV were de novo in 22/23 patients. One patient carried compound heterozygous PV in the UBA5 gene (Table 1, Figures 1B–D, 2A, Appendix S2).

In patients with EMAtS, 19% (10/52) had PV/LPV laying on eight different genes, namely *SCN2A* ($n=3$), *STX1B* ($n=1$), *CSNK2B* ($n=1$), *NEDD4L* ($n=1$), *SLC2A1* ($n=1$), *SLC6A1* ($n=1$), *KCTD7* ($n=1$), and *ASAH1* ($n=1$, Table 1, Figures 1B–D, 2A, Appendix S2). All variants are de novo, except those on *ASAH1* and *KCTD7*, which were biallelic.

In patients with DS, we identified PV/LPV in 78% (40/51) affecting *SCN1A* ($n=35$), *KCNA2* ($n=2$), and *GABRB3* ($n=1$). We identified two patients with clustering *PCDH19* epilepsy due to de novo *PCDH19* PV (Table 1, Figures 1B–D, 2A, Appendix S2). In 30 patients, PV/LPV were de novo, in 3 inherited from unaffected heterozygous parents, and in two inherited from an unaffected parent carrying the variant in low somatic mosaicism. In 5 cases, segregation study could not be realized.

In DEE-SWAS, 14% of patients carried de novo PV/LPV in *GRIN2A* (3/21). In 4/11 patients with LGS, de novo causal variants were identified in *CHD2* ($n=2$), *RORB* ($n=1$), and *SCN2A* ($n=1$). Finally, among five children with HHE, one had a de novo *SCN1A* LPV and one de novo *CACNA1A* LPV (Table 1, Figures 1B–D, 2A, Appendix S2).

3.3.2 | Other phenotypes

In patients with benign (familial) neonatal/infantile seizures, PV/LPV were identified in 82% (32/39) laying on *KCNQ2* ($n=20$), *PRRT2* ($n=11$), and *SCN2A* ($n=1$). In four patients, a recurrent deletion including *KCNQ2*, *CHRNA4*, and *EEF1AE* was identified. In three patients, we identified a heterozygous *PRRT2* deletion. PV/LPV were de novo in eight patients, inherited from an affected parent in 5, inherited from a healthy parent in 15. In three cases, we could not perform a segregation study (Table 1, Figures 1C, 2B, Appendix S2).

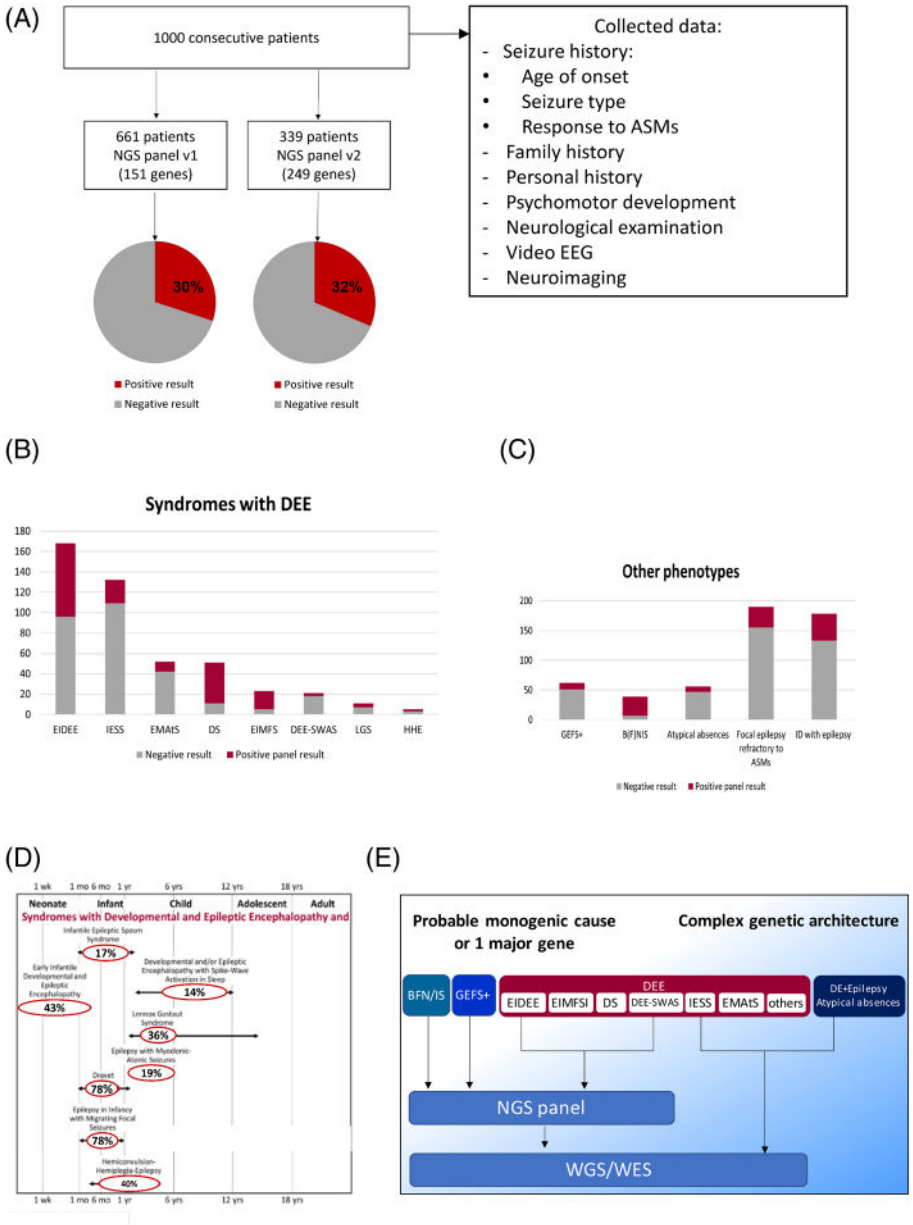

FIGURE 1 Description of the 1000 included patients and their NGS results. (A) NGS results obtained in this cohort and study design, (B) NGS results in patients with developmental epileptic encephalopathies, and (C) NGS result in patients with other epilepsy syndromes according to ILAE classification. (D) Genetic results in different DEE according to the ILAE classification (Adapted from Wirrel et al.[4]). (E) Proposed algorithm for genetic testing in patients with pediatric epilepsy. DEE-SWAS, developmental and epileptic encephalopathy with spike–wave activity in sleep; DS, Dravet syndrome; EIDEE, Early-infantile developmental and epileptic encephalopathy syndrome; EIMFS, epilepsy of infancy with migrating focal seizures; EMAtS, epilepsy with myoclonic-atonic seizures; GEFS+, generalized epilepsy with febrile seizures plus; HHE, hemiconvulsion hemiplegia epilepsy syndrome; IESS, infantile epileptic spasms syndrome; LGS, Lennox–Gastaut syndrome.

We tested 62 patients with GEFS+ and found LPV/PV in 16% (10/62) affecting *SCN1A* ($n=7$), *HCN1* ($n=1$), *SCN1B* ($n=1$), and *STX1B* ($n=1$). PV/LPV were inherited from a heterozygous affected parent in 3, inherited from a heterozygous unaffected parent in 2, and de novo in 3. For two patients, segregation could not be performed (Table 1, Figures 1C, 2B, Appendix S2).

Fifty-six children presented GGE with atypical absences. In this group, we found PV/LPV in 9/56 (16%) affecting five different genes, namely *SLC2A1* ($n=3$), *NIPA2* ($n=3$), *NRXN1* ($n=1$), *NUS1* ($n=1$), and *SYNGAP1* ($n=1$). In the three patients with *NIPA2* deletion, CGH-array confirmed the recurrent 15q11.2 BP1-BP2 deletion. PV/LPV were de novo in five patients, inherited from a

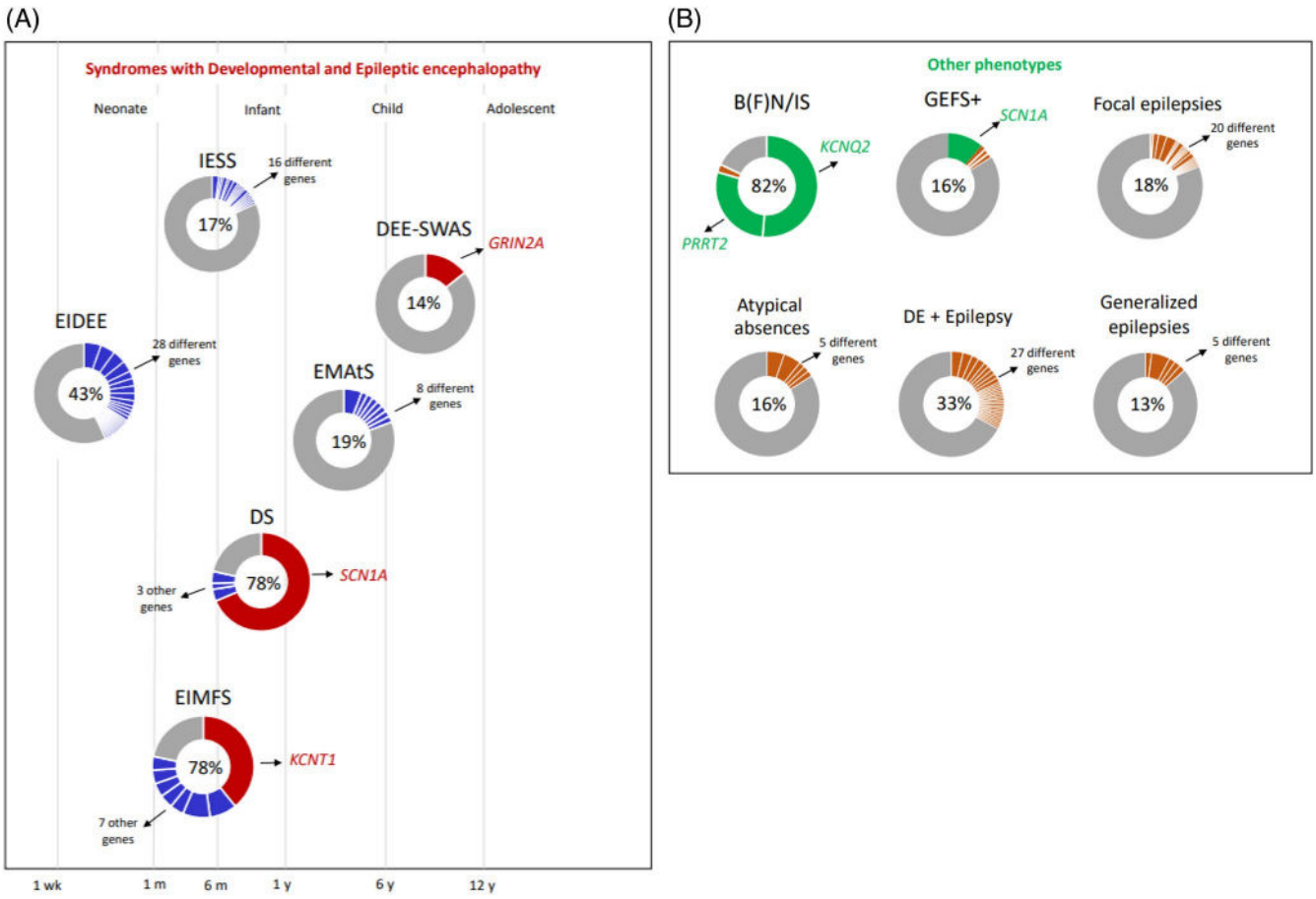

FIGURE 2 Genetic results obtained in this cohort in patients with developmental epileptic encephalopathies and other epilepsy syndromes according to ILAE classification. (A) The graphs show the percentage of molecular diagnosis obtained in each epilepsy syndrome that are presented from left to right following the age at onset (Adapted from Wirrel et al.[4]). We shaded in red the percentage of patients with a pathogenic variant on one major gene (indicated by an arrow). We shaded in blue the percentage of patients with pathogenic variants in heterogeneous genes. In gray, we indicate the percentage of patients with a negative panel result. In the center, we present the percentage of patients with a confirmed genetic etiology. (B) The graphs show the percentage of molecular diagnosis obtained in syndromes other than DEEs. We shaded in green the percentage of patients with a pathogenic variant in one major gene (indicated by arrows). We shaded in orange the percentage of patients with pathogenic variants in more heterogeneous genes. In gray, we indicate the percentage of patients with a negative panel result. In the center, we present the percentage of patients with a confirmed genetic etiology. DEE-SWAS, developmental and epileptic encephalopathy with spike–wave activity in sleep; DS, Dravet syndrome; EIDEE, Early-infantile developmental and epileptic encephalopathy syndrome; EIMFS, epilepsy of infancy with migrating focal seizures; EMAtS, epilepsy with myoclonic-atonic seizures; GEFS+, generalized epilepsy with febrile seizures plus; IESS, infantile epileptic spasms syndrome.

heterozygous unaffected parent in 1, inherited from a heterozygous affected parent in 1, and inherited from an unaffected parent carrying the variant in somatic mosaicism in one. A segregation study could not be done for one patient (Table 1, Figures 1C, 2B, Appendix S2).

In 52 patients with non-classified GGE, we identified PV/LPV in 7 (13%) on 5 different genes, namely *SLC2A1* ($n=3$), *SCN8A* ($n=1$), *HCN1* ($n=1$), *EPMA2* ($n=1$), and *CSNK2B* ($n=1$, Table 1, Figures 1C, 2B, Appendix S2).

In the group of focal epilepsies, a genetic etiology was established in 35/190 patients (18%) and affected 20 different genes. In particular, PV/LPV were identified in *SCN8A* ($n=6$), *PRRT2* ($n=4$), *DEPDC5* ($n=3$), *NPRL3* ($n=3$), *PCDH19* ($n=3$), *MTOR* ($n=2$), *GABRA1*, *SETBP1*, *SCN2A*, *NRXN1*, *SLC2A1*, *SYN1*, *PNPO*, *RELN*, *NPRL2*, *GABRG2*, *KCNC2*, *LGI1*, *DYRK1A*, and *KCNT1* ($n=1$ each). For 19 patients, PV/LPV were de novo, inherited from a heterozygous healthy parent in 11, inherited from an affected father carrying the variant in somatic mosaicism in 1, and from an affected heterozygous parent in 1. One patient carried *PNPO* compound heterozygous variants. For two patients, segregation analyses could not be performed (Table 1, Figures 1C, 2B, Appendix S2).

In 138 patients with developmental encephalopathies with seizures started after the age of 2 years, we identified PV/LPV in 45 (33%) affecting 27 different genes. They are de novo in 34 patients. Autosomal recessive conditions were identified in four patients (*CNTNAP2* in three

and *PNKP* in one). In one patient, the recurrent 15q11.2 BP1-BP2 deletion was identified. In two brothers, we found a duplication of *SCN1A* and *SCN2A* that was absent in their parents, suggesting a gonadal mosaicism (Table 1, Figures 1C, 2B, Appendix S2).

4 | DISCUSSION

We report the detailed diagnostic yield of a TNGS panel in a series of 1000 consecutive individuals referred to our reference center for rare epilepsy. The overall diagnostic yield in this cohort is 31%. In the literature, the diagnostic yield of TNGS panels, whole exome sequencing (WES) and whole genome sequencing (WGS), defined as the percentage of "solved" cases, varies between 1% and 89%. This variability has been attributed to differences in the genetic strategies (i.e., panels targeting a variable number and selection of genes, WES, or WGS) and in the interpretation/classification of variants, especially prior to the publication of guidelines for molecular data interpretation.[1,2,5,6]

In this series, we did not find a relevant difference in the diagnostic yield of a first panel version targeting 151 genes (30%) compared to a second panel version targeting 249 genes (32%). Although this is important at an individual level, it may not support this approach from a cost–benefit and timeline perspective for such rare genes. This is consistent with literature data showing that a "core" panel of 30 genes accounts for the majority of diagnoses, while larger panels contribute a small percentage of additional diagnoses.[1]

Early studies evaluating genetic testing for epilepsies focused on the efficacy of TNGS approaches in large patient cohorts but often provided limited clinical descriptions. Many studies did not report detailed phenotypes or included cohorts with mixed phenotypes, comprising both monogenic and non-monogenic epilepsies with different syndromes and ages of onset. This complicates the assessment of the impact of specific phenotypic features on test yield.[1] However, the variable diagnostic yield is primarily influenced by the population included in the reported cohorts (i.e., pediatric versus adult epilepsies, focal epilepsies, DEE, metabolic, or structural epilepsies).[1]

We analyzed our targeted NGS results based on the patients' electroclinical diagnosis according to the ILAE classification.[4] Our findings highlight the significant role of epilepsy types and syndromes in determining the diagnostic rate of TNGS strategies, as shown in more recent studies.[1,2]

In our series, patients with DEE had an overall diagnostic yield of 37%. Examining the results in more detail, we achieved a genetic diagnosis in 2/3 of patients with EIMFS and DS. These syndromes are monogenic diseases linked to a few major genes: *SCN1A* for DS and *KCNT1* for EIMFS. For patients with EIDEE, we attained a molecular diagnosis in nearly half of the patients with contributions from three major genes (*STXBP1*, *SCN2A*, and *KCNQ2*).

In contrast, only 17% of patients with IESS and 19% of those with EMAtS received a molecular diagnosis through our panel, with no major gene identified in these groups. This low yield in IESS may be partly due to the exclusion of patients with structural etiologies, such as tuberous sclerosis, from this series. Additionally, genetic heterogeneity has been reported in patients with EMAtS, suggesting a multifactorial inheritance pattern in most cases.[7,8]

Our results suggest that TNGS remains relevant for patients with BFN/IS. Patients with DEE, particularly those with EIMFS and DS, are also good candidates for first-step TNGS. The high number of diagnoses made by TNGS in these groups of patients suggests that a Mendelian cause underlies these epilepsies and that WES or WGS could be useful in patients with a negative TNGS result (Figure 1E).

For patients with IESS or EMAtS, the low diagnostic yield from the panel may be due to a high genetic heterogeneity warranting proposing WES or WGS as the initial step in the diagnosis genetic work-up, where available (Figure 1E). However, the low diagnostic yield in both syndromes may also be related to a complex inheritance that cannot be fully resolved by WES or WGS.

Interestingly, for DEE-SWAS, although the yield is not high, the causal role of one major gene, *GRIN2A*, may support the use of TNGS as a first step.[9] Finally, patients with focal epilepsies, atypical absences, not-classified drug resistant GGE, and developmental encephalopathy with epilepsy onset after 2 years show a high genetic heterogeneity and have a low diagnostic yield achieved by panel orienting toward the hypothesis of a complex inheritance. WES or WGS as a first option for genetic testing could allow a molecular diagnosis in some cases (Figure 1E). The development of polygenic risk score models could be relevant to understanding the complex genetic architecture of these syndromes.[10]

Genetic testing for patients with epilepsy has evolved, and a variety of testing options are now available within the diagnostic framework, including TNGS panels, WES, and WGS. However, clear guidelines regarding the optimal testing strategy are still lacking, and the recommendations should include the availability of the different methodologies and the cost benefit.

A major limitation of TNGS compared to WES and WGS is the restricted number of genes analyzed. Negative results with TNGS will necessitate additional testing and increase the delay of the diagnosis delivery. Nevertheless, TNGS offers deeper sequencing than WES and WGS, providing greater confidence in the results for variants in

known epilepsy genes, especially when those variants are already classified as pathogenic.[1]

In our cohort, the depth of the sequencing in TNGS enabled the detection of variants at low levels of mosaicism. Mosaicism is believed to be an underreported cause of genetic disorders, with Stosser et al. reporting its presence in 3.5% of 893 patients.[11] We identified probable low-level mosaicism variants in 11 patients (3.5%) and in 6 parents, which has significant implications for genetic counseling. An additional advantage of TNGS is that testing known genes can expedite genetic analysis, particularly when "rapid panels" with accelerated turnaround times are developed by diagnostic laboratories, allowing diagnoses in < 1 week.

The epilepsy and epilepsy syndrome diagnosis impacts the yield of TNGS and allows a rational approach in many monogenic syndromes.

WGS is a comprehensive genetic testing approach that can identify variants in non-coding regions, repeat expansions, copy number variants (CNVs), structural variants, uniparental disomy, and mitochondrial DNA variants. However, the analysis of WGS results is challenging due to the generation of variants of uncertain significance (VUS) and the need for functional studies on newly identified genes not previously associated with epilepsy. Moreover, WGS is not yet widely available in routine clinical practice and often remains a research tool in many settings.[12] Our patients with negative results are entering a genome diagnosis program, and we will evaluate the yield of this program in the coming years.

CGH-array enables the identification of pathogenic copy number variations (CNVs) in approximately 5%–16% of patients with epilepsy, particularly when associated with neurodevelopmental disorders.[1] CGH-array remains a viable option where WGS is not available, serving as a second-line diagnostic test, especially for patients with neurodevelopmental disorders.[13] In our cohort, we identified 22 exonic deletions or duplications. Notably, by targeting the gene *NIPA2* located in the 15q11.2 region, we found the recurrent 15q11.2 BP1-BP2 deletion in six patients, which was further characterized by CGH. Allowing the detection of both CNVs and structural variants, WGS is replacing CGH-array.

Our genetic testing algorithm for pediatric epilepsy recommends using the NGS panel as a first step for patients with BFI/NS, EIDEE, EIMFS, Dravet syndrome, DEE-SWAS, and GEFS+. While the number of patients studied by WGS remains limited, preventing a definite change in our stepwise genetic testing approach, our findings along with existing literature[1,2] suggest that WES or WGS could be considered as a first genetic test, when available, for patients with IESS, EMAtS, developmental encephalopathies with seizure onset after age 2, atypical absences, and not-classified genetic generalized epilepsy with seizures refractory to anti-seizure medications (ASMs).

Our results suggest that refining the phenotype and utilizing multidisciplinary discussion of genetic testing results can enhance diagnostic yield. Identifying the spectrum of phenotypes associated with epilepsy genes will facilitate the interpretation of genetic results. When proposing genetic testing, epilepsy should not be viewed as a single disorder. Utilizing the ILAE epilepsy syndrome classification is valuable for guiding the genetic diagnostic strategy for patients, enabling targeted testing through panels in certain monogenic epilepsies or those linked to major genes.

In conclusion, genetic testing has now become an essential tool in the diagnosis of epilepsy and may play a crucial role in some syndromes. A close collaboration between epileptologists and Health authorities should consider the cost of genetic testing as part of the work-up for patients with epilepsy, alongside neuroimaging and other assessments. Furthermore, employing the ILAE classification will enhance the comparison of genetic studies and improve our understanding of the genetic contributions to various epilepsy syndromes.

ACKNOWLEDGMENTS

This work is supported by the Chair Geen-DS funded by the FAMA fund hosted by the Swiss Philanthropy Foundation. RN is supported by ANR-23-RHUS-0002 (France 2030). We acknowledge the use of the bioresources of the Necker Imagine DNA biobank (BB-033-00065).

CONFLICT OF INTEREST STATEMENT

None of the authors has any conflict of interest to disclose. 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.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Giulia Barcia https://orcid.org/0000-0001-6657-5040
Monika Eisermann https://orcid.org/0000-0003-3634-2101
Anna Kaminska https://orcid.org/0000-0003-3805-6651

REFERENCES

1. Sheidley BR, Malinowski J, Bergner AL, Bier L, Gloss DS, Mu W, et al. Genetic testing for the epilepsies: a systematic review. Epilepsia. 2022;63(2):375–87.

2. Castellotti B, Ragona F, Freri E, Messina G, Magri S, Previtali R, et al. Next-generation sequencing in pediatric-onset epilepsies: analysis with target panels and personalized therapeutic approach. Epilepsia Open. 2024;9(5):1922–30.
3. Splinter K, Adams DR, Bacino CA, Bellen HJ, Bernstein JA, Cheatle-Jarvela AM, et al. Effect of genetic diagnosis on patients with previously undiagnosed disease. N Engl J Med. 2018;379(22):2131–9.
4. Wirrell EC, Nabbout R, Scheffer IE, Alsaadi T, Bogacz A, French JA, et al. Methodology for classification and definition of epilepsy syndromes with list of syndromes: report of the ILAE task force on nosology and definitions. Epilepsia. 2022;63(6):1333–48.
5. Stefanski A, Calle-López Y, Leu C, Pérez-Palma E, Pestana-Knight E, Lal D. Clinical sequencing yield in epilepsy, autism spectrum disorder, and intellectual disability: a systematic review and meta-analysis. Epilepsia. 2021;62(1):143–51.
6. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J. 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(5):405–24.
7. Tang S, Addis L, Smith A, Topp SD, Pendziwiat M, Mei D, et al. Phenotypic and genetic spectrum of epilepsy with myoclonic atonic seizures. Epilepsia. 2020;61(5):995–1007.
8. Routier L, Verny F, Barcia G, Chemaly N, Desguerre I, Colleaux L, et al. Exome sequencing findings in 27 patients with myoclonic-atonic epilepsy: is there a major genetic factor? Clin Genet. 2019;96(3):254–60.
9. Samanta D. GRIN2A-related epilepsy and speech disorders: a comprehensive overview with a focus on the role of precision therapeutics. Epilepsy Res. 2023;189:107065.
10. Stevelink R, Luykx JJ, Lin BD, Leu C, Lal D, Smith AW, et al. Shared genetic basis between genetic generalized epilepsy and background electroencephalographic oscillations. Epilepsia. 2021;62(7):1518–27.
11. Stosser MB, Lindy AS, Butler E, Retterer K, Piccirillo-Stosser CM, Richard G, et al. High frequency of mosaic pathogenic variants in genes causing epilepsy-related neurodevelopmental disorders. Genet Med. 2018;20(4):403–10.
12. Chowdhury SR, Whitney R, RamachandranNair R, Bijarnia Mahay S, Sharma S. Genetic testing in pediatric epilepsy: tools, tips, and navigating the traps. Pediatr Neurol. 2024;157:42–9.
13. Baer S, Schalk A, Miguet M, Schaefer É, El Chehadeh S, Ginglinger E, et al. Copy number variation and epilepsy: state of the art in the era of high-throughput sequencing—a multicenter cohort study. Pediatr Neurol. 2024;159:16–25.

SUPPORTING INFORMATION

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

How to cite this article: Barcia G, Chemaly N, Gobin-Limballe S, Losito E, Aubart M, Sarda E, et al. Genetic etiologies with a large NGS panel in a monocentric cohort of 1000 patients with pediatric onset epilepsies. Epilepsia Open. 2025;10:1065–1073. https://doi.org/10.1002/epi4.70057