Author/s:
Scheffer, IE;Bennett, CA;Gill, D;de Silva, MG;Boggs, K;Marum, J;Baker, N;Palmer, EE;Howell, KB;Andrews, I;Antony, J;Ardern-Holmes, S;Bye, AM;Cardamone, M;Chelakkadan, S;Clark, D;Curnow, SR;Dabscheck, G;Fahey, MC;Freeman, JL;Gupta, S;Harvey, AS;Hildebrand, MS;Inder, M;Kanhangad, M;Kornberg, AJ;Kothur, K;Lawson, JA;Leventer, RJ;Malone, S;Menezes, MP;Mohammad, S;Nagarajan, L;Pillai, S;Pridmore, C;Procopis, PG;Sampaio, H;Silberstein, J;Sinclair, A;Smith, N;Subramanian, G;Troedson, C;Ware, T;White, SM

Title:
Exome sequencing for patients with developmental and epileptic encephalopathies in clinical practice

Date:
2023-01-01

Citation:
Scheffer, I. E., Bennett, C. A., Gill, D., de Silva, M. G., Boggs, K., Marum, J., Baker, N., Palmer, E. E., Howell, K. B., Andrews, I., Antony, J., Ardern-Holmes, S., Bye, A. M., Cardamone, M., Chelakkadan, S., Clark, D., Curnow, S. R., Dabscheck, G., Fahey, M. C. ,... White, S. M. (2023). Exome sequencing for patients with developmental and epileptic encephalopathies in clinical practice. Developmental Medicine and Child Neurology, 65 (1), pp.50-57. https://doi.org/10.1111/dmcn.15308.

Persistent Link:
https://hdl.handle.net/11343/316391

Received: 21 December 2021 | Accepted: 2 May 2022

DOI: 10.1111/dmcn.15308

ORIGINAL ARTICLE

Exome sequencing for patients with developmental and epileptic encephalopathies in clinical practice

Ingrid E. Scheffer[1,2,3,4] | **Caitlin A. Bennett**[1] | **Deepak Gill**[5] | **Michelle G. de Silva**[6,7] | **Kirsten Boggs**[7,8] | **Justine Marum**[6] | **Naomi Baker**[2,6] | **Australian Genomics DEE Flagship**[†] | **Elizabeth E. Palmer**[9] | **Katherine B. Howell**[2,4,10]

[1]Epilepsy Research Centre, Department of Medicine, Austin Health, The University of Melbourne, Heidelberg, Victoria

[2]Department of Paediatrics, The University of Melbourne, Victoria

[3]Florey Institute of Neuroscience and Mental Health, Heidelberg, Victoria

[4]Murdoch Children's Research Institute, Parkville, Victoria

[5]TY Nelson Department of Neurology and Neurosurgery, The Children's Hospital at Westmead, New South Wales

[6]Victorian Clinical Genetics Services, Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Victoria

[7]Australian Genomics Health Alliance, Melbourne

[8]Sydney Children's Hospitals Network, Sydney

[9]Clinical Genetics Service, Sydney Children's Hospital, Randwick, New South Wales

[10]Department of Neurology, The Royal Children's Hospital, Parkville, Victoria, Australia

Correspondence
Ingrid Scheffer, Epilepsy Research Centre, University of Melbourne, Level 2, Melbourne Brain Centre, 245 Burgundy St, Heidelberg, Victoria 3084, Australia.
Email: scheffer@unimelb.edu.au

Funding information
National Health and Medical Research Council, 1091593, 1104831 and 1113531; University of Melbourne; Murdoch Children's Research Institute; University of Sydney

Abstract

Aim: To assess the clinical utility of exome sequencing for patients with developmental and epileptic encephalopathies (DEEs).

Method: Over 2 years, patients with DEEs were recruited for singleton exome sequencing. Parental segregation was performed where indicated.

Results: Of the 103 patients recruited (54 males, 49 females; aged 2 weeks–17 years), the genetic aetiology was identified in 36 out of 103 (35%) with management implications in 13 out of 36. Exome sequencing revealed pathogenic or likely pathogenic variants in 30 out of 103 (29%) patients, variants of unknown significance in 39 out of 103 (38%), and 34 out of 103 (33%) were negative on exome analysis. After the description of new genetic diseases, a molecular diagnosis was subsequently made for six patients or through newly available high-density chromosomal microarray testing.

Interpretation: We demonstrate the utility of exome sequencing in routine clinical care of children with DEEs. We highlight that molecular diagnosis often leads to changes in management and informs accurate prognostic and reproductive counselling. Our findings reinforce the need for ongoing analysis of genomic data to identify the aetiology in patients in whom the cause is unknown. The implementation of genomic testing in the care of children with DEEs should become routine in clinical practice.

Abbreviations: CNV, Copy number variant; DEE, Developmental and epileptic encephalopathy; VUS, Variants of unknown significance.

Ready access to genomic testing for severe diseases is only just becoming a reality in most parts of the world, including Australia. This partly relates to affordability and access to molecular testing, expertise in analysis, and increasing availability through Medicare-funded items.

In epilepsy, gene discovery has led to insights into underlying mechanisms and inheritance of seizure disorders, transforming clinical diagnosis and management. Within the epilepsies, gene discovery has had the greatest impact in the most severe group of epilepsies called the developmental and epileptic encephalopathies (DEEs). The DEEs are severe epilepsies that typically begin in infancy or childhood. The DEEs are characterized by frequent seizures, prominent epileptiform abnormalities on electroencephalography (EEG), and developmental slowing or regression.[1] Most children have multiple seizure types and are at risk of a range of comorbidities including intellectual disability, depression, sleep problems, autism spectrum disorder, movement disorders, and gastrointestinal problems. The developmental abnormality relates to both the underlying aetiology and the impact of the superimposed epileptic encephalopathy.

DEEs occur in approximately 1 in 1000 individuals, equating to approximately 300 newly diagnosed children in Australia per year.[2,3] The advent of next generation sequencing has resulted in an aetiological diagnosis in up to 50% of patients with DEEs where a pathogenic variant is identified via molecular studies, including gene panels, exome, and genome studies.[2–8] While the DEEs are highly genetically heterogeneous with more than 400 genes implicated, each individual genetic disease is rare. Genetic mutations often arise de novo and follow dominant inheritance; however, some patients have autosomal recessive, X-linked, or mitochondrial inheritance.[9] We aimed to trial exome sequencing in the clinical setting, engaging a network of paediatric neurologists around Australia, to determine the utility of exome sequencing in clinical practice.

What this paper adds

- The cause was identified in 35% of patients with developmental and epileptic encephalopathies.
- *KCNQ2*, *CDKL5*, *SCN1A*, and *STXBP1* were the most frequently identified genes.
- Reanalysis of genomic data found the cause in an additional six patients.
- Genetic aetiology was identified in 41% of children with seizure onset under 2 years, compared to 18% with older onset.
- Finding the molecular cause led to management changes in 36% of patients with DEEs.

METHOD

The DEE Flagship of the Australian Genomics Health Alliance was formed in 2016 and included all regions of Australia with flagship leads in each state. All paediatric neurologists were asked to identify patients according to the following criteria: (1) patient first seen by the referring neurologist in 2016 or later; (2) patient younger than 18 years at recruitment; (3) frequent seizures (frequency not specified); (4) epileptiform abnormalities on EEG; (5) developmental delay and/or regression (excluding children with static intellectual disability and epilepsy); (6) magnetic resonance imaging without a causative finding, such as an acquired disorder (e.g. perinatal insult), or aetiology suggesting a specific genetic disorder, such as tuberous sclerosis complex. However, malformations such as focal cortical dysplasia could be included; and (7) normal microarray or a non-diagnostic finding on microarray.

The referring paediatric neurologist completed a referral form (Appendix S1) which was reviewed by a recruitment committee of three paediatric epileptologists. Once approved as fulfilling inclusion criteria, the family then received genetic counselling. Parents or legal guardians provided written informed consent for study participation and publication of results.

Exome sequencing was performed on blood-derived DNA by a clinically accredited laboratory (Victorian Clinical Genetics Services, Melbourne). Coding regions were enriched using either the Sureselect QXT CREv1 or CREv2 exome capture kit (Agilent Technologies, Santa Clara, CA, USA) and sequencing was performed on an Illumina (San Diego, CA, USA) instrument, with a mean coverage of 100x and a minimum of 90% bases with 15x sequencing coverage. Targeted analysis was performed on an epilepsy gene panel of 365 genes (Appendix S2). When no pathogenic variant was identified, the analysis examined all genes associated with human disease. Variants were analysed using in silico prediction tools PolyPhen, SIFT, and CADD, and whether they were recurrent. Molecular findings were discussed at a multidisciplinary meeting and classified as Class 5 (pathogenic), Class 4 (likely pathogenic), or Class 3 (variants of unknown significance [VUS]), as per American College of Medical Genetics guidelines.[10] Where possible, segregation was performed to determine if the variant was inherited or had arisen de novo. The exome results were provided to families by their neurologist or a genetic counsellor.

This study was approved by the Human Research Ethics Committee of Melbourne Health.

RESULTS

There were 127 patients referred to the Australian Genomics DEE Flagship. Of these, 15 did not satisfy inclusion criteria for the following reasons: a causative copy number variant (CNV) on microarray in two, no microarray in four, no magnetic resonance imaging in three, no epileptiform activity on EEG in two, no developmental delay or regression in one, prior genetic testing in one, and presentation before 2016 in two. A further nine patients were approved but either declined participation or were transferred to alternative Australian Genomics programmes.

In total, 103 patients (54 male, 52%), aged 2 weeks to 17 years, underwent exome sequencing. Age of seizure onset was under 2 years in 75 (73%) and 2 years or older in 28 (27%) (range 1 day–14 years). Seizure frequency was daily in 88 (85%), weekly or multiple weekly in nine (9%), monthly in one (1%), and unknown in three (3%). A further two (2%) patients with epilepsy-aphasia syndromes: Landau–Kleffner syndrome and epilepsy with continuous spike-and-wave during sleep, did not have clinical seizures recorded. Epilepsy syndrome diagnoses at presentation were Ohtahara syndrome (n = 1), early myoclonic encephalopathy (n = 1), early-infantile DEE (n = 14), infantile spasms (n = 38), Dravet syndrome (n = 4), early-onset absence epilepsy (n = 2), myoclonic-atonic epilepsy (n = 8), Landau–Kleffner syndrome (n = 3), epilepsy with continuous spike-and-wave during sleep (n = 1), atypical childhood epilepsy with centrotemporal spikes (n = 1), Lennox–Gastaut syndrome (n = 2), late-onset spasms (n = 1), neurodegenerative disease (n = 1), and DEE unclassified (n = 26). Development was abnormal at presentation in 29, normal in 55 (all of whom had subsequent plateau or regression), unclear or unknown in six, and too early to tell in 13 patients with seizure onset in the first few days or weeks of life.

Pathogenic or likely pathogenic variants were identified in 30 out of 103 (29%) individuals (Table 1), 39 out of 103 (38%) had VUS, and 34 out of 103 (33%) had no potentially pathogenic variants identified. Parental segregation was performed via Sanger sequencing in 23 out of 30 patients with pathogenic or likely pathogenic variants to determine the inheritance pattern. Twenty-one had de novo pathogenic variants (X-linked or autosomal dominant), one had compound heterozygous autosomal recessive pathogenic variants, and one was maternally inherited with Sanger sequencing suggesting mosaicism. The level of mosaicism in the mother was then quantified in a research laboratory using droplet digital polymerase chain reaction and shown to be 10.5% in blood.

Since completion of the study in December 2018, the genetic cause has been identified in a further six patients (Table 1). In three patients, VUS were reclassified as pathogenic with additional data. In one child, a homozygous variant in *SMPD4* was reclassified after a recent publication describing a new developmental syndrome.[11] The other child had two variants in *PIGN*: a recurrent heterozygous deletion across the intron-exon boundary was initially considered pathogenic[12] and a heterozygous missense variant has now been reclassified as pathogenic; the phenotype is consistent with *PIGN* encephalopathy. On segregation studies, the *PIGN* deletion was maternally inherited and the missense variant paternally inherited. The third patient had a homozygous variant in *NRROS* and was included in a recent paper reporting this gene as causing a severe infantile-onset neurodegenerative disease.[13]

A causative CNV was identified in the remaining three patients. In one, a newly available high-density microarray detected a small deletion missed on standard microarray, which included the gene *STXBP1*. A second patient had a duplication of exons 4 to 15 in *CDKL5* identified on a new Invitae gene panel using an in-house algorithm for exonic CNV detection. The duplication was missed on both standard exome sequencing and microarray analysis (not high-density) and fits well with the patient's phenotype. In the remaining patient, a CNV of uncertain significance was upgraded to pathogenic after the recent publication of a case series showing that this CNV was pathogenic.[14]

Pathogenic variants were more likely to be found in children with earlier seizure onset. The genetic aetiology was identified in 31 out of 75 (41%) with seizure onset under the age of 2 years compared with 5 out of 28 (18%) with seizure onset over the age of 2 years.

Of the 36 out of 103 patients with an identified genetic aetiology, 13 diagnoses carried management implications. These included treatment of the underlying biochemical abnormality (*SLC2A1* [n = 1]), choice of antiseizure medication (*KCNQ2* [n = 4], *SCN1A* [n = 3], *SCN8A* [n = 2], *SCN2A* [n = 1]), choice of other medication (*ATP1A3* [n = 1]), and screening for disease-related complications (*COL4A1* [n = 1]).

DISCUSSION

Yield of exome sequencing in Australia-wide cohort of DEEs

Exome sequencing is transforming diagnosis in human disease. This is especially relevant to the severe epilepsies known as the DEEs. In this prospective, Australia-wide study, we aimed to integrate exome sequencing into clinical practice. We identified the genetic aetiology in 36 out of 103 (35%) patients. We found 33 out of 36 patients had single nucleotide variants causing missense, stopgain, or frameshift mutations, and 3 out of 36 had CNVs which were identified as pathogenic through the course of this study.

Of our cohort, 30 out of 103 (30%) remain of unknown aetiology and 37 out of 103 (36%) have VUS. VUS should be reinterrogated over time as variant classification may change with larger cohorts or interaction through online exchanges such as Matchmaker Exchange.[15] For example, patient 21 had a *SCN1A* missense VUS and was in foster care, so parental segregation was not possible. In the last year, another patient with Dravet syndrome and this exact *SCN1A* variant was published,[16] elevating the classification of variant pathogenicity from uncertain to a recurrent pathogenic variant, without the need for parental segregation.

Our findings highlight the importance of reanalysis in patients with DEEs who are negative on initial molecular testing. Reanalysis has previously been highlighted as useful for patients with DEEs of unknown cause by Rochtus et al.;[17] however, only half of their patients had had prior exome sequencing performed 7 years earlier. Our data show that, even with recent negative exome sequencing, regular and timely reanalysis can be fruitful with the frequent discovery of new DEE genes. Reanalysis of the single nucleotide variants in exome data as new DEE genes (e.g.

TABLE 1 Epilepsy syndrome diagnosis in patients with developmental and epileptic encephalopathies (DEEs) and pathogenic or likely pathogenic variants

Patient	Sex; age at seizure onset	Epilepsy syndrome	Genetic finding			SIFT, PolyPhen, CADD scores	gnomAD
			Gene	Variant	Inheritance		
1	F; 5 months	Infantile spasms	*ALG13*	c.320A > G, p.Asn107Ser	De novo	D (0), B (0.093), 2.795	Absent
2	M; 1 days	Early myoclonic encephalopathy	*ARX*	c.196G > A, p.Gly66Ser	De novo	T (0.11), B (0.314), 3.745	Absent
3	F; 2 days	Early-infantile DEE[a]	*ATP1A3*	c.2443G > A, p.Glu815Lys	De novo	D (0.01), D (0.991), 3.562	Absent
4	F; 6 months	DEE unclassified	*CDKL5*	c.539C > T, p.Pro180Leu	De novo	D (0), D (0.995), 3.888	Absent
5	F; 6 months	Epileptic spasms without hypsarrhythmia	*CDKL5*	c.786C > A, p.Tyr262*	Unknown[b]	-, –, 4.545	Absent
6	M; 5 days	Early-infantile DEE	*CDKL5*	c.89G > A, p.Cys30Tyr	De novo	D (0), P (0.676), 4.268	Absent
7	F; 3 months	Early-infantile DEE	*COL4A1*	c.3656G > T, p.Gly1219Val	Unknown[b]	D (0), P (0.997), 3.342	Absent
8	M; 12 months	DEE unclassified	*GABRG2*	c.529C > T, p.Arg177*	De novo	---	Absent
9	F; 6 months	DEE, Dravet-like syndrome	*HCN1*	c.1139 T > A, p.Ile380Asn	De novo	D (0), D (0.954), 4.173	Absent
10	M; 8 months	Infantile spasms	*HECW2*	c.3597C > G, p.Asn1199Lys	De novo	D (0.01), D (0.986), 3.202	Absent
11	M; 3 months	Early-infantile DEE	*KCNA2*	c.1120A > G, p.Thr374Ala	De novo	D (0), D (0.995), 3.879	Absent
12	M; 3 days	Early-infantile DEE	*KCNQ2*	c.1678C > T, p.Arg560Trp	Unknown[b]	D (0), D (1), 4.037	Absent
13	F; 3 days	Early-infantile DEE	*KCNQ2*	c.746 T > C, p.Leu249Pro	De novo	D (0), P (0.819), 4.079	Absent
14	M; 1 days	Early-infantile DEE	*KCNQ2*	c.860C > T, p.Thr287Ile	De novo	D (0), D (0.979), 3.841	Absent
15	F; 2 days	Ohtahara syndrome	*KCNQ2*	c.881C > T, p.Ala294Val	De novo	D (0), P (0.821), 3.632	Absent
16	M; 14 years	DEE unclassified	*MECP2*	c.1083_1102del, p.Lys363Serfs*23	De novo	---	Absent
17	F; 2 years 9 months	Neurodegenerative disease	*MECP2*	Complex variant	De novo	---	---
18	F; 3 years	Myoclonic-atonic epilepsy	*NEXMIF*	c.438C > A, p.Cys146*	De novo	---	Absent
19	M; 5 years	Lennox–Gastaut syndrome	*PPP3CA*	c.1283dupC, p.Thr429Asnfs*22	De novo	---	Absent
20	M; 3 months	Dravet syndrome	*SCN1A*	c.1159_1162delCTTT, p.Leu387Ilefs*3	Unknown[b]	---	Absent
21	F; 9 months	Dravet syndrome	*SCN1A*	c.193 T > G; p.Tyr65Asp (recurrent)	Unknown[b]	D (0), D (0.997), 4.098	Absent
22	F; 8 months	Dravet syndrome	*SCN1A*	c.5536_5539delAAAC, p.Lys1846Serfs*11	Inherited from mosaic mother who had FS+	---	Absent
23	F; 5 weeks	Early-infantile DEE	*SCN2A*	c. 5018 T > C, p.Ile1673Thr	De novo	D (0), P (0.89), 3.977	Absent
24	F; 3 months	DEE unclassified	*SCN8A*	c.4400 T > G, p.Phe1467Cys	De novo	D (0), P (0.999), 4.521	Absent
25	F; 3 years 10 months	DEE unclassified	*SCN8A*	c.4877G > A, p.Arg1626His	De novo	D (0), P (0.862), 4.325	Absent

TABLE 1 (Continued)

Patient	Sex; age at seizure onset	Epilepsy syndrome	Genetic finding			SIFT, PolyPhen, CADD scores	gnomAD
			Gene	Variant	Inheritance		
26	M; 5 months	Early-onset absence epilepsy[c] with regression	*SLC2A1*	c.618delinsTT, p.Cys207Leufs*30	Unknown[b]	---	Absent
27	F; 21 months	Early-onset absence epilepsy	*SLC6A1*	c.331G > A, p.Gly111Arg	De novo	D (0), D (1), 4.239	Absent
28	M; 1–2 weeks	Early-infantile DEE	*STXBP1*	c.794 + 1G > A (recurrent)	De novo	---	Absent
29	M; 3 months	Infantile spasms	*STXBP1*	c.901delC, p.Gln301Argfs*7	Unknown[b]	---	Absent
30	F; 17 days	Early-infantile DEE	*WWOX*	c.864G > A, p.Trp288* (heterozygous deletion on other allele)	Compound heterozygous	---	Absent
Patients in whom the aetiology was identified by reanalysis or other methods							
31	F; 9 months	DEE unclassified	*PIGN*	c.548_549 + 6del (maternal), c.2237 T > G, p.Ile746Arg (paternal)	Compound heterozygous	--- D (0), P (0.562), 3.655	50 hets, 0 hom (1.27e-4) 3 hets, 0 hom (1.21e-5)
32	M; 3 months	DEE unclassified	*SMPD4*	c.2491_2492delCT, p.Leu831Valfs*215	Homozygous	---	Absent
33	F; 12 months	Epileptic spasms (not West syndrome)	*NRROS*	c.1981delC, p.Leu661Serfs*97	Homozygous	---	Absent
34	F; 2 months	Infantile spasms	9q33.3q34.11 deletion including *STXBP1*, high density microarray		De novo	---	---
35	M; 11 months	DEE unclassified	16p13.3 deletion (approx. 0.4 Mb, clinical microarray)		De novo	---	---
36	F; 7 days	Early-infantile DEE	*CDKL5*	Duplication of exons 4–15	Unknown	---	Absent

Abbreviations: CADD, unscaled C-scores; FS+, febrile seizures plus; gnomAD, v2 and v3 combined; hets, heterozygotes; hom, homozygotes; PolyPhen: B = benign, P = possibly damaging, D = probably damaging; SIFT, T = tolerated, D = deleterious.

[a]Early-infantile DEE: onset 3 months or younger.

[b]Segregation testing not possible.

[c]Early-onset absence epilepsy: onset <4 years.

SMPD4, NRROS) are identified will increase the yield over time. Also, there is increasing recognition of the overlap in genetic aetiologies across neurological disorders such as neurodevelopmental, autism spectrum, and psychiatric disorders.[18] CNVs of uncertain significance may be subsequently shown to be pathogenic, as in the male with a 16p13.3 deletion.[14] Furthermore, the recent development of higher density microarrays means that pathogenic CNVs missed on routine clinical microarrays may be identified, such as the female with a CNV including *STXBP1*. High-density microarrays are likely to become the standard of care in future. Other strategies include trio exome sequencing to identify de novo or recessive pathogenic variants, and higher depth of sequencing to find mosaic pathogenic variants.

Novel genetic mechanisms are also likely to explain the DEEs in individuals in whom the aetiology is still unknown. For example, genome sequencing enables the identification of intronic variants such as poison exons and repeat expansions, which have recently been recognised as causing epilepsy.[19,20] Another promising level of genetic analysis involves epigenetic studies with recent findings of episignatures that aid in identification of underlying pathogenic variants.[21] Inheritance, even in seemingly monogenic DEEs, is likely to be complex, with recent evidence showing a higher burden of ultrarare pathogenic variants in patients with a known pathogenic variant of major effect.[22] In addition, common genetic variation, studied through polygenic risk scores based on genome wide association studies, is also likely to influence phenotypic variability in the DEEs.

Benefits of finding the genetic aetiology

Bringing exome sequencing into routine clinical care is transformational for patients and families as it ends the diagnostic odyssey, which has hitherto taken many years and resulted in considerable frustration and distress for families. Although the yield in our study was higher for patients with infantile-onset DEEs, the yield in patients with older onset was still valuable. Notably, discovery of the aetiology at any age, including older adults, is important, as it often carries management implications which can improve outcome.[23]

Genetic diagnosis often has management implications, as we found in almost 40% of our patients in whom the aetiology was identified. Diagnosis informs choice of antiseizure medicine which, by improving seizure control, may improve long-term outcome and reduce the mortality risk. It also enables the cessation of antiseizure medications that may be exacerbating seizures, such as carbamazepine in Dravet syndrome.[23] Molecular diagnosis also informs the risk of specific comorbidities, such as autism spectrum disorder, gait, speech, and sleep disorders. Features associated with specific genetic diseases may not emerge until adult life. For example, in *PCDH19* clustering epilepsy, a quarter of adolescents and adult females developed psychosis, highlighting the need for early recognition and management.[24] For some families, the genetic diagnosis of a rare disease with an extremely poor prognosis may lead to redirection to palliative care.

Genetic findings often provide critical information for accurate reproductive counselling to young parents considering further children. A major issue is the risk of parental mosaicism, as illustrated by patient 22 with Dravet syndrome. The patient's mother had a history of febrile seizures plus and was 10.5% mosaic for the *SCN1A* pathogenic variant. The finding of parental mosaicism is critical as it carries an increased recurrence risk for future pregnancies. We previously showed that 8% of probands with DEEs due to a dominant mutation had a parent who was mosaic on deep sequencing for their child's pathogenic variant, placing the parent at significant risk of having a second affected child.[25]

These major molecular advances come with new challenges such as the family's grief at a serious diagnosis so early in their child's life. The ready availability of online resources means it is hard to protect families from the often devastating consequences of these disorders. Conversely, in some diseases such as *KCNQ2* DEE, seizures may remit in the first few years of life which may offer reassurance.[26] This highlights the urgent need for more support for families who are facing such a difficult journey.

Relationship of epilepsy syndrome to genetic aetiology

Our cohort showed a wide range of epilepsy syndromes. Genetic DEEs are associated with specific epilepsy syndromes and our patients often presented with the classical syndromology of their disease. For example, all four patients with *KCNQ2*-DEE presented in the first week of life with tonic seizures and profound developmental impairment.[26] One had Ohtahara syndrome with burst-suppression pattern on EEG, while three had early-infantile DEE. Our three patients with *STXBP1* pathogenic variants, including one who had a deletion of *STXBP1*, presented with early-infantile DEE with onset under 3 months or infantile spasms, as typically described.[27] There is increasing recognition that overlap of different epilepsy syndromes may be seen with specific genetic aetiologies, such as early onset absence epilepsy and myoclonic-atonic epilepsy in *SLC6A1*,[28] and early onset absence epilepsy, epilepsy with eyelid myoclonias, and myoclonic-atonic epilepsy in *NEXMIF*.[29]

In our cohort, the genes most frequently identified were *KCNQ2* ($n = 4$), *CDKL5* ($n = 4$), *SCN1A* ($n = 3$), *STXBP1* ($n = 3$), *SCN8A* ($n = 2$), and *MECP2* ($n = 2$), with other genes implicated in single cases (Table 1). These genes have also been seen in larger cohort studies such as Epi4K where the most frequently identified genes differed somewhat to our findings,[30] or in larger cohorts using gene panels.[7,31] Notably, our exome analysis interrogated more genes (365) than in previous studies.[7,8] Where a pathogenic variant was not identified, we then expanded the analysis to all genes associated with human disease. Our first insights into the genetic

epidemiology of the DEEs have been gleaned from Victorian and Scottish studies,[2,4,32] but we require larger numbers of patients to understand the epidemiology of each rare genetic DEE, given that there are now more than 400 genes.

Implementation in clinical practice

Our programme shows that the implementation of exome sequencing in clinical practice is both feasible and worthwhile. In addition to the significant patient- and family-centred benefits, there are also health economic benefits from avoiding the need for further aetiological investigations.[2,3] These data are being used to support an application for health system funding for routine exome sequencing in patients with DEEs.

The discovery of a pathogenic variant allows families to join international groups focused on their child's specific genetic disease. They can also join parent groups that exist to support families and help them with the journey toward precision medicine. The benefit of these groups cannot be overestimated, as shown by the growth of Genetic Epilepsy Team Australia, an inspiring group of Australian parents whose vision is to develop precision medicines for all individuals with DEEs.

The remarkable advances in molecular genetics have led to an aetiological diagnosis in many patients with severe epilepsies. While our study of exome sequencing study found the cause in 35% of children, ongoing molecular diagnosis is critical in solving the remaining cases. All molecular data should be analysed from a broad perspective, including all genes relevant to neurological diseases, given the increasing overlap in the genetic basis of these disorders. Clinical implementation of exome sequencing is feasible now and, with the introduction of newer technologies such as genome sequencing, there is the real promise of finding the cause in all patients with DEEs. Early genetic diagnosis informs targeted management, and means that Australian patients are poised for emerging precision medicine trials.

ACKNOWLEDGMENTS

This work was supported by an NHMRC Program Grant (1091593), Senior Practitioner Fellowship (1104831) and NHMRC grant 1113531, which also supports Australian Genomics. The Murdoch Children's Research Institute was supported by the Victorian State Government Operational Infrastructure Support Program.

The members of the Australian Genomics DEE Flagship who referred patients to the study and contributed clinical data are as follows: Ian Andrews,[1] Jayne Antony,[2] Simone Ardern-Holmes,[2] Ann M Bye,[1] Michael Cardamone,[1] Shabeed Chelakkadan,[3] Damian Clark,[4] Sarah R Curnow,[5,7] Gabriel Dabscheck,[5,7] Michael C Fahey,[3] Jeremy L Freeman,[5,7] Sachin Gupta,[2] A Simon Harvey,[5,6,7] Michael S Hildebrand,[7,8] Marie Inder,[8] Manoj Kanhangad,[3] Andrew J Kornberg,[5] Kavitha Kothur,[2] John A Lawson,[1] Richard J Leventer,[5,6,7] Stephen Malone,[9] Manoj P Menezes,[2] Shekeeb Mohammad,[2,10] Lakshmi Nagarajan,[11] Sekhar Pillai,[1] Clair Pridmore,[4] Peter G Procopis,[2] Hugo Sampaio,[1] Jonathon Silberstein,[11] Adriane Sinclair,[9] Nicholas Smith,[4] Gopinath Subramanian,[12] Christopher Troedson,[2] Tyson Ware,[13] Susan M White.[7]

1 Department of Neurology, Sydney Children's Hospital, Randwick, New South Wales; 2 TY Nelson Department of Neurology and Neurosurgery, The Children's Hospital at Westmead, New South Wales; 3 Neurology Department, Monash Children's Hospital, Clayton, Victoria; 4 Department of Neurology and Clinical Neurophysiology, Women's and Children's Hospital, North Adelaide, South Australia; 5 Department of Neurology, The Royal Children's Hospital, Parkville, Victoria; 6 Department of Paediatrics, The University of Melbourne, Victoria; 7 Murdoch Children's Research Institute, Parkville, Victoria; 8 Epilepsy Research Centre, Department of Medicine, Austin Health, The University of Melbourne, Heidelberg, Victoria; 9 Department of Neurosciences, Queensland Children's Hospital, South Brisbane, Queensland; 10 Children's Hospital at Westmead Clinical School, Faculty of Medicine and Health, University of Sydney, Sydney, New South Wales; 11 Department of Neuroscience and Neurology, Perth Children's Hospital, Nedlands, Western Australia; 12 Department of Neurology, John Hunter Children's Hospital, New South Wales; 13 Department of Paediatrics, Royal Hobart Hospital, Hobart, Tasmania, Australia. Open access publishing facilitated by The University of Melbourne, as part of the Wiley - The University of Melbourne agreement via the Council of Australian University Librarians.

DATA AVAILABILITY STATEMENT

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

ORCID

Ingrid E. Scheffer https://orcid.org/0000-0002-2311-2174
Deepak Gill https://orcid.org/0000-0002-6219-9479

REFERENCES

1. Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, et al. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. Epilepsia. 2017;58(4):512–21.
2. Howell KB, Eggers S, Dalziel K, Riseley J, Mandelstam S, Myers CT, et al. A population-based cost-effectiveness study of early genetic testing in severe epilepsies of infancy. Epilepsia. 2018;59(6):1177–87.
3. Palmer EE, Schofield D, Shrestha R, Kandula T, Macintosh R, Lawson JA, et al. Integrating exome sequencing into a diagnostic pathway for epileptic encephalopathy: Evidence of clinical utility and cost effectiveness. Mol Genet Genomic Med. 2018;6(2):186–99.
4. Symonds JD, Elliott KS, Shetty J, Armstrong M, Brunklaus A, Cutcutache I, et al. Early childhood epilepsies: epidemiology, classification, aetiology, and socio-economic determinants. Brain. 2021;144(9):2879–91.
5. Na JH, Shin S, Yang D, Kim B, Kim HD, Kim S, et al. Targeted gene panel sequencing in early infantile onset developmental and epileptic encephalopathy. Brain Dev. 2020;42(6):438–48.
6. Hamdan FF, Myers CT, Cossette P, Lemay P, Spiegelman D, Laporte AD, et al. High Rate of Recurrent *De Novo* Mutations in

Developmental and Epileptic Encephalopathies. Am J Hum Genet. 2017;101(5):664–85.
7. Heyne HO, Artomov M, Battke F, Bianchini C, Smith DR, Liebmann N, et al. Targeted gene sequencing in 6994 individuals with neurodevelopmental disorder with epilepsy. Genet Med. 2019;21(11):2496–503.
8. Willimsky EK, Munzig A, Mayer K, Biskup S, Abicht A, Hoertnagel K, et al. Next Generation Sequencing in Pediatric Epilepsy Using Customized Panels: Size Matters. Neuropediatrics. 2021;52(2):92–7.
9. McTague A, Howell KB, Cross JH, Kurian MA, Scheffer IE. The genetic landscape of the epileptic encephalopathies of infancy and childhood. Lancet Neurol. 2016;15(3):304–16.
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(5):405–24.
11. Magini P, Smits DJ, Vandervore L, Schot R, Columbaro M, Kasteleijn E, et al. Loss of *SMPD4* Causes a Developmental Disorder Characterized by Microcephaly and Congenital Arthrogryposis. Am J Hum Genet. 2019;105(4):689–705.
12. Fleming L, Lemmon M, Beck N, Johnson M, Mu W, Murdock D, et al. Genotype-phenotype correlation of congenital anomalies in multiple congenital anomalies hypotonia seizures syndrome (MCAHS1)/*PIGN*-related epilepsy. Am J Med Genet A. 2016;170A(1):77–86.
13. Dong X, Tan NB, Howell KB, Barresi S, Freeman JL, Vecchio D, et al. Bi-allelic LoF *NRROS* Variants Impairing Active TGF-beta1 Delivery Cause a Severe Infantile-Onset Neurodegenerative Condition with Intracranial Calcification. Am J Hum Genet. 2020;106(4):559–69.
14. Mucha BE, Banka S, Ajeawung NF, Molidperee S, Chen GG, Koenig MK, et al. A new microdeletion syndrome involving *TBC1D24*, *ATP6V0C*, and *PDPK1* causes epilepsy, microcephaly, and developmental delay. Genet Med. 2019;21(5):1058–64.
15. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat. 2015;36(10):928–30.
16. 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(6):1234–9.
17. Rochtus A, Olson HE, Smith L, Keith LG, El Achkar C, Taylor A, et al. Genetic diagnoses in epilepsy: The impact of dynamic exome analysis in a pediatric cohort. Epilepsia. 2020;61(2):249–58.
18. Li J, Cai T, Jiang Y, Chen H, He X, Chen C, et al. Genes with *de novo* mutations are shared by four neuropsychiatric disorders discovered from NPdenovo database. Mol Psychiatry. 2016;21(2):290–7.
19. Carvill GL, Engel KL, Ramamurthy A, Cochran JN, Roovers J, Stamberger H, et al. Aberrant Inclusion of a Poison Exon Causes Dravet Syndrome and Related *SCN1A*-Associated Genetic Epilepsies. Am J Hum Genet. 2018;103(6):1022–9.
20. Corbett MA, Kroes T, Veneziano L, Bennett MF, Florian R, Schneider AL, et al. Intronic ATTTC repeat expansions in *STARD7* in familial adult myoclonic epilepsy linked to chromosome 2. Nat Commun. 2019;10(1):4920.
21. Aref-Eshghi E, Bend EG, Colaiacovo S, Caudle M, Chakrabarti R, Napier M, et al. Diagnostic Utility of Genome-wide DNA Methylation Testing in Genetically Unsolved Individuals with Suspected Hereditary Conditions. Am J Hum Genet. 2019;104(4):685–700.
22. Epi25 Collaborative. Ultra-Rare Genetic Variation in the Epilepsies: A Whole-Exome Sequencing Study of 17,606 Individuals. Am J Hum Genet. 2019;105(2):267–82.
23. Catarino CB, Liu JY, Liagkouras I, Gibbons VS, Labrum RW, Ellis R, et al. Dravet syndrome as epileptic encephalopathy: evidence from long-term course and neuropathology. Brain. 2011;134(Pt 10):2982–3010.
24. Vlaskamp DRM, Bassett AS, Sullivan JE, Robblee J, Sadleir LG, Scheffer IE, et al. Schizophrenia is a later-onset feature of *PCDH19* Girls Clustering Epilepsy. Epilepsia. 2019;60(3):429–40.
25. 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(17):1646–8.
26. Weckhuysen S, Mandelstam S, Suls A, Audenaert D, Deconinck T, Claes LR, et al. *KCNQ2* encephalopathy: emerging phenotype of a neonatal epileptic encephalopathy. Ann Neurol. 2012;71(1):15–25.
27. Stamberger H, Nikanorova M, Willemsen MH, Accorsi P, Angriman M, Baier H, et al. *STXBP1* encephalopathy: A neurodevelopmental disorder including epilepsy. Neurology. 2016;86(10):954–62.
28. Johannesen KM, Gardella E, Linnankivi T, Courage C, de Saint Martin A, Lehesjoki AE, et al. Defining the phenotypic spectrum of *SLC6A1* mutations. Epilepsia. 2018;59(2):389–402.
29. Stamberger H, Hammer TB, Gardella E, Vlaskamp DRM, Bertelsen B, Mandelstam S, et al. *NEXMIF* encephalopathy: an X-linked disorder with male and female phenotypic patterns. Genet Med. 2021;23(2):363–73.
30. Epi4K Consortium, Epilepsy Phenome/Genome Project, Allen AS, Berkovic SF, Cossette P, Delanty N, et al. *De novo* mutations in epileptic encephalopathies. Nature. 2013;501(7466):217–21.
31. Carvill GL, Heavin SB, Yendle SC, McMahon JM, O'Roak BJ, Cook J, et al. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in *CHD2* and *SYNGAP1*. Nat Genet. 2013;45(7):825–30.
32. Howell KB, Freeman JL, Mackay MT, Fahey MC, Archer J, Berkovic SF, et al. The severe epilepsy syndromes of infancy: A population-based study. Epilepsia. 2021;62(2):358–70.

SUPPORTING INFORMATION

The following additional material may be found online:
Appendix S1 Australian Genomic Health Alliance patient referral form.
Appendix S2 Australian Genomics DEE Flagship gene list.

How to cite this article: Scheffer IE, Bennett CA, Gill D, de Silva MG, Boggs K, Marum J, Australian Genomics DEE Flagship, et al. Exome sequencing for patients with developmental and epileptic encephalopathies in clinical practice. Dev Med Child Neurol. 2022;00:1–8. https://doi.org/10.1111/dmcn.15308