Epidemiology and etiology of infantile developmental and epileptic encephalopathies in Tasmania

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Abstract
We sought to determine incidence, etiologies, and yield of genetic testing in infantile onset developmental and epileptic encephalopathies (DEEs) in a population isolate, with an intensive multistage approach. Infants born in Tasmania between 2011 and 2016, with seizure onset <2 years of age, epileptiform EEG, frequent seizures, and developmental impairment, were included. Following review of EEG databases, medical records, brain MRIs, and other investigations, clinical genetic testing was undertaken with subsequent research interrogation of whole exome sequencing (WES) in unsolved cases. The incidence of infantile DEEs was 0.44/1000 per year (95% confidence interval 0.25 to 0.71), with 16 cases ascertained. The etiology was structural in 5/16 cases. A genetic basis was identified in 6 of the remaining 11 cases (3 gene panel, 3 WES). In two further cases, WES identified novel variants with strong in silico data; however, paternal DNA was not available to support pathogenicity. The etiology was not determined in 3/16 (19%) cases, with a candidate gene
Infantile developmental and epileptic encephalopathies (DEEs) have a range of etiologies including >60 genetic causes, and in many patients, the cause remains unknown. The infantile DEEs comprise a group of epilepsy syndromes with infantile spasms (West syndrome) typically accounting for over half the cases. \(^1,2\) The incidence of DEEs overall was estimated as 0.27/1000 births in the North London study \(^1\) and as 0.54/1000 per year in our recent Victorian study; \(^2\) the difference was probably related to methodology as each had different inclusion criteria and only those with a specific syndrome were counted in the North London estimate. Reliable epidemiological data for the infantile DEEs is required for health services planning and will inform the need for genetic testing in the diagnosis and management in these severe epilepsies.

Genetic investigations have revolutionized understanding of the causes of DEEs. This knowledge has begun to be implemented in the clinic, predominantly using gene panel testing, and diagnostic yields are growing as new discoveries are made. \(^3\) Incorporating whole exome sequencing (WES) into the diagnostic protocol of DEEs increases the yield and reduces overall costs associated with reaching a diagnosis. \(^2\)

The ascertainment process is shown in Figure S1. We screened >1200 EEG records and identified 89 patients with epileptiform features. Seventy-three cases were excluded; 40 due to age or date of onset; 24 had a mild or self-limited epilepsy syndrome; 4 had normal developmental outcome; and 5 had acute symptomatic seizures (four with neonatal hypoxic-ischemic encephalopathy and one with traumatic brain injury).

Clinical details obtained included developmental history, seizure semiology, and comorbidities from interview, review of medical records, and a validated seizure questionnaire. \(^4\) EEG findings, neuroimaging, and metabolic investigations were reviewed. Specific epilepsy syndromes were diagnosed where possible.

Research WES was conducted using Agilent SureSelect XT Human All Exon + UTR v5 (75 Mb) kit and 100 bp paired-end sequencing on the HiSeq 2500 System. Trio WES analysis including both parents was performed to allow segregation of variants, where possible. WES read mapping, alignment processing, and variant calling were performed using GATK best practices. \(^5\) Initially, a panel of 423 candidate genes was interrogated from the WES for single nucleotide variants and, if this was negative, exome-wide analysis was performed. Ultrarare variants predicted to result in functional changes and segregating with affected status were validated by Sanger sequencing. Plausible connections to epilepsy or neurodevelopmental conditions were investigated by review of the literature and performing functional studies (reported separately; \(^6\) Berecki et al, in submission). Pathogenicity of variants was assessed according to ACMG criteria. \(^7\) Data from unsolved cases were regularly re-reviewed at 6- to 12-month intervals. Ethical approval was obtained from the Human Research and Ethics Committee (Tasmania) Network (Reference H0013627).

Sixteen patients met the criteria for incident infantile DEEs in the six-year period. All were of Anglo-Australian
background. All patients were identified from review of EEG records or by pediatric neurologists servicing Tasmania. Correspondence with pediatricians around the state did not identify any additional patients.

Of the 16 patients, 5 were male. Clinical and molecular findings are summarized in Table 1. Twelve had abnormal development prior to seizure onset or as newborn infants. Median seizure onset was 6 months (range 3 days to 20 months). Six patients had West syndrome with infantile spasms.

There were 36,408 births in Tasmania in 2011-2016 with net migration in this period being negligible. The incidence of infantile DEEs and infantile spasms in Tasmania was 0.44/1000 (95% confidence interval 0.25 to 0.71) per year and 0.17/1000 (95% confidence interval 0.06 to 0.36) per year, respectively.

At ascertainment, five infants had an established etiology based on history and neuroimaging (Table 1). Cases 1 and 2 had lissencephaly including one with a causative copy number variant (17p13.3 Miller-Dieker microdeletion) and the second with a mosaic microduplication at 17p regarded as likely pathogenic. One patient had focal cortical dysplasia, and one had clinically confirmed tuberous sclerosis (genetic testing not performed). Case 5 had extensive unilateral cystic encephalomalacia, consistent with a large perinatal anterior circulation infarct. As this patient presented with infantile spasms, she met our inclusion criteria despite the acquired cause. There were no metabolic etiologies identified in our cohort.

The etiology was unknown at ascertainment in the remaining 11 patients (cases 6-16); however, three subsequently had positive clinical genetic testing. Two had de novo KCNQ2 variants, and one had an intronic change in ARX resulting in retention of intron 4 and predicted early termination of the ARX protein; \(^6\) the variant segregated with autism spectrum disorder in his mildly affected mother and brother.

Three patients had pathogenic or likely pathogenic variants identified on research genetic testing in DHDDS, GABRB2 (reported in \(^5\)), and CACNA1G. For case 12 with a variant in CACNA1G, in vitro electrophysiological evaluation showed a pathogenic gain of function (Berecki et al, in submission). Cases 9 and 13, with heterozygous variants in SCN8A and SNAP25, respectively, were regarded as likely solved, as their clinical patterns were consistent with the literature, and there was strong in silico data. However, as both were novel variants and parental DNA was unavailable for segregation, the ACMG classification for these two cases remained “uncertain significance.”

Three cases were regarded as currently unsolved. Case 14 had compound heterozygous variants in FAT1; segregation analysis confirmed one variant was inherited from each parent. Both variants were regarded as damaging on in silico analysis, but the gene is not established as a DEE gene, so the variants were regarded as of uncertain significance.

Etiology for two patients remains unknown despite detailed review of exome-wide variants. For one, trio data are available, but for the other parental DNA was not available. Further clinical descriptions and variant details are given in the supporting information.

**4 | DISCUSSION**

The incidence of the infantile DEEs in Tasmania (0.44/1000 per year) is consistent with estimates from North London, UK, and Victoria, Australia.\(^1\),\(^2\) Our incidence of infantile spasms (0.17/1000 per year) was at the lower end of previous estimates, which ranged from 0.2-0.45/1000 per year.\(^1\),\(^2\),\(^10\),\(^11\) The Victorian study also included patients with acquired brain injuries (12% of the cohort), whereas these patients were excluded from our study with the exception of one who had infantile spasms (an automatic inclusion).

The etiology of the epilepsy was definitively identified in 11 of our 16 patients (69%). Five had a major structural abnormality. Genetics was important in this group with a defined genetic etiology in the two lissencephaly cases and a presumed but unstudied genetic abnormality in the tuberous sclerosis case.

Of the nonstructural cases, 6/11 had a definite genetic etiology. If we include cases 9 and 13, where the identified genes were highly plausible in terms of pathogenicity (SCN8A, SNAP25), but the absence of proof of a de novo etiology precluded strict ACMG classification as pathogenic, then the diagnostic success rate for the whole cohort climbs to 81% (Figure 1). Although our sample size was small, this high success rate in identifying the etiology can be attributed in part to the intensive scrutiny of WES data. This included trio testing where possible, consideration of various modes of inheritance including mosaicism and repeated interrogation incorporating newly published data into our analysis after standard clinical testing. Our rigorous testing identified genetic etiologies in two cases that were negative on initial exome-wide analysis, as has also been shown to be valuable in other studies of unsolved DEE cases.\(^1\),\(^2\),\(^13\)

The reported diagnostic yield of genetic testing in infantile DEEs depends on the methods used, the inclusion criteria of the sample population, and whether “solved” patients with prior testing have been excluded. Recent studies generally find that 25%-50% of cases are solved.\(^2\),\(^14\)–\(^17\) although one study of a selected group of 14 cases studied by trio whole genome sequencing claimed diagnostic findings in all.\(^18\) Our findings provide a “real-world” estimate reflecting that parental samples are not always obtainable from an epidemiologically ascertained cohort.
Strengths of our study are that the cohort was ascertained by comprehensive review of all EEG recordings performed in Tasmania from 2009 to 2016 and contact with all pediatricians and neurologists caring for children. The data are thus likely to be complete, which is supported by our incidence estimates being in broad agreement with others. It is possible, but unlikely,

| Subject number/Gender | Onset age of seizures | Clinical details | Etiology |
|-----------------------|-----------------------|-----------------|----------|
|                       | Sydrome               |                 |          |
| 1/F                   | 8 mo                  | Epileptic spasms| Lissencephaly |
|                       | West syndrome         | Profound GDD    | Miller-Dieker 17p13.3 microdeletion |
|                       |                       | EEG: Hypsarrhythmia |          |
|                       |                       | MRI: Moderate lissencephaly, gradient: posterior more severe than anterior |          |
| 2/M                   | 7 mo                  | Spasms, focal motor seizures| Lissencephaly |
|                       | West syndrome         | Profound GDD    | 17p mosaic microduplication |
|                       |                       | EEG: Hypsarrhythmia |          |
|                       |                       | MRI: Moderate lissencephaly, gradient: posterior more severe than anterior, pontine hypoplasia |          |
| 3/M                   | 20 mo                 | Unifocal seizures| Focal cortical dysplasia |
|                       | DEE                   | Regression with seizures. Surgery curative. | Genetic testing not done |
|                       |                       | Mild language delay |          |
|                       |                       | EEG: Left frontotemporal IEDs |          |
|                       |                       | MRI: Segmental focal cortical dysplasia, subependymal nodules |          |
| 4/F                   | 2 wk                  | Focal tonic, FIAS| Tuberous sclerosis complex |
|                       | DEE                   | Plateau with seizures. Mild language delay. | Genetic testing not done |
|                       |                       | EEG: Multifocal IEDs. |          |
|                       |                       | MRI: Multifocal tubers. |          |
| 5/F                   | 5 mo                  | Spasms, Focal motor seizures| Antnatal elastic vascular |
|                       | West syndrome         | Hemiplegia, regression with spasms | Genetic testing not done |
|                       |                       | EEG: Hypsarrhythmia; unifocal centro-temporal spike IEDs |          |
|                       |                       | MRI: Antenatal venous infarction with multicystic encephalomalacia |          |
| 6/F                   | 3 d                   | Focal seizures, migrating focal seizures| KCNQ2 |
|                       | EIMFS                 | Mild GDD        | c.637C>T p.Arg213Trpa |
|                       |                       | EEG: Ictal rhythms migrating between hemispheres; 6 mo & 13 mo normal | pathogenic |
|                       |                       | MRI: Normal     | de novo |
| 7/F                   | 2 mo                  | Focal tonic seizures, spasms, multifocal myoclonia| KCNQ2 |
|                       | West syndrome         | Acquired microcephaly, dyskinesia, profound GDD | c.593G>A p.Arg198Gln |
|                       |                       | EEG: Hypsarrhythmia; multifocal discharges | pathogenic |
|                       |                       | MRI: Acquired moderate cerebral atrophy | de novo |
| 8/M                   | 4 wk                  | Tonic-clonic seizures, focal tonic seizures| ARX |
|                       | DEE                   | Severe GDD      | c.1449-1 G>C p.Leu484<sup>a</sup> |
|                       |                       | EEG: Bilateral occipital IEDs | pathogenic |
|                       |                       | MRI: Hypoplastic corpus callosum |          |
| 9/F                   | 6 mo                  | Tonic-clonic seizures, FBTC| SCN8A |
|                       | DEE                   | Language delay  | c. 5009T>G p.Met1670Arg |
|                       |                       | EEG: 11 mo-Ictal rhythm midline to frontocentral regions; 2 y1 mo-GSW, PSW | uncertain significance |
|                       |                       | MRI: Normal     | (de novo status unproven) |
that patients with infantile DEEs may have been missed if they had not come to the attention of the mainstream medical profession or are cared for solely by a general practitioner, especially in the remote areas of Tasmania. Also, our combined clinical and research genetic approach resulted in a very high yield.

A weakness of our study is the small sample size; thus, while our global estimates are robust, we cannot provide estimates of the frequency of individual syndromes or genes. Indeed, based on established estimates of the incidence of Dravet syndrome, one to two cases of Dravet syndrome might have been expected in our cohort.\textsuperscript{19} The lack of Dravet cases in our cohort is most likely because they will not have satisfied inclusion criteria. It is likely that their EEG is normal in the first 2 years of life, seizures

| Subject number/Gender | Onset age of seizures | Syndrome | Clinical details | Etiology |
|-----------------------|-----------------------|----------|-----------------|----------|
| 10/ F 5 y 10 mo       | 6 mo                  | DEE      | Absence with eyelid myoclonia, absence, eyelid myoclonia, myoclonic jerks, tonic-clonic seizures, NCSE. Profound GDD, visual impairment. EEG: Marked photosensitivity, 3-4Hz GSW, PSW, myoclonic-atonic seizure. MRI: Normal | DHDDS c.632G>A p.Arg211Gln\textsuperscript{a} de novo Pathogenic\textsuperscript{b} |
| 11/ M deceased 17 d   | 5 d                   | EME      | Myoclonic jerks. Decreased activity, poor feeding, jitteriness. EEG: Burst suppression. MRI: Normal | GABRB2 c.851C>A p.Thr284Lys\textsuperscript{c} de novo Likely pathogenic\textsuperscript{b} |
| 12/ F 8 y 3 mo        | 7 mo                  | DEE      | Febrile seizures, vibratory tonic seizures, tonic-clonic seizures, absence. Profound GDD, hypotonia, truncal ataxia, ambulating with walker at 4 y. EEG: GSW, multifocal discharges, PSW. MRI: Normal, no cerebellar atrophy. | CACNA1G c.2727G>C p.Leu909Phe\textsuperscript{c} Likely pathogenic (gain of function in vitro [unpublished]; de novo status unproven)\textsuperscript{b} |
| 13/ F 4 y 7 mo        | 5 mo                  | West syndrome | Spasms. Remission at 8 mo, severe ID and GDD, seizure free without medication. EEG: Hypsarrhythmia. MRI: Normal | SNAP25 c. 526C>T p.Arg176Cys\textsuperscript{c} Uncertain significance\textsuperscript{b} (de novo status unproven) |
| 14/ F 4 y 10 mo       | 13 mo                 | DEE      | Myoclonic jerks; Focal motor seizures at 2 y. Hypotonia, delayed visual maturation, severe ID and GDD. EEG: Multifocal discharges, normal at 23 mo. MRI: Normal. | Unknown Candidate gene: FAT1 c.8626G>C p.Asp2876His\textsuperscript{c} c.7655A>G p.Glu2552Gly\textsuperscript{c} Uncertain significance\textsuperscript{b} |
| 15/ M 4 y 6 mo        | 9 mo                  | West syndrome | Spasms. Developmental plateau with spasms. EEG: Hypsarrhythmia. MRI: Normal | Unknown |
| 16/ F 6 y 9 mo        | 10 mo                 | DEE      | Focal tonic seizures, tonic-clonic seizures, FIAS. Specific learning difficulties. EEG: 15 mo-normal; 21 mo-occipital ictal rhythm; 22 mo-occipital IEDs; 4 y-normal. MRI: Normal | Unknown |

Abbreviations: BS, burst suppression; DEE, developmental and epileptic encephalopathy; EIMFS, epilepsy of infancy with migrating focal seizures; EME, early myoclonic encephalopathy; FBTC, focal to bilateral tonic-clonic seizure; FIAS, focal impaired awareness seizure; GDD, global developmental delay; GSW, generalized spike-wave; ID, intellectual disability; IEDs, interictal epileptiform discharges; MFDs, multifocal discharges; NCSE, nonconvulsive status epilepticus; PSW, polyspike-wave.

\textsuperscript{a} previously published variant.

\textsuperscript{b} ACMG classification.

\textsuperscript{c} Novel variant.
may be infrequent in infancy and always associated with fever (therefore diagnosed as febrile seizures), and developmental decline may not yet be apparent. Whole genome sequencing studies may reveal pathogenic variants not identified by WES.

Our hypothesis-free, intensive, multistage approach to genetic testing identified pathogenic or likely pathogenic variants in a number of DEE genes. Our findings directly informed diagnosis, treatment, and prognostic planning for these infants and enabled accurate reproductive counseling for their parents.

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CONFLICTS OF INTEREST

None of the authors have 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.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

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