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Date:
2024-11-01

Citation:
Viswanathan, S., Oliver, K. L., Regan, B. M., Schneider, A. L., Myers, C. T., Mehaffey, M. G., LaCroix, A. J., Antony, J., Webster, R., Cardamone, M., Subramanian, G. M., Chiu, A. T. G., Roza, E., Teleanu, R. I., Malone, S., Leventer, R. J., Gill, D., Berkovic, S. F., Hildebrand, M. S. ,... Scheffer, I. E. (2024). Solving the Etiology of Developmental and Epileptic Encephalopathy with Spike–Wave Activation in Sleep (D/EE-SWAS). Annals of Neurology, 96 (5), pp.932-943. https://doi.org/10.1002/ana.27041.

Persistent Link:
https://hdl.handle.net/11343/351048

Solving the Etiology of Developmental and Epileptic Encephalopathy with Spike–Wave Activation in Sleep (D/EE-SWAS)

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Objective: To understand the etiological landscape and phenotypic differences between 2 developmental and epileptic encephalopathy (DEE) syndromes: DEE with spike–wave activation in sleep (DEE-SWAS) and epileptic encephalopathy with spike–wave activation in sleep (EE-SWAS).

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.27041

Received Feb 23, 2024, and in revised form May 31, 2024. Accepted for publication Jul 11, 2024.

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Additional supporting information can be found in the online version of this article.

Methods: All patients fulfilled International League Against Epilepsy (ILAE) DEE-SWAS or EE-SWAS criteria with a Core cohort (n = 91) drawn from our Epilepsy Genetics research program, together with 10 etiologically solved patients referred by collaborators in the Expanded cohort (n = 101). Detailed phenotyping and analysis of molecular genetic results were performed. We compared the phenotypic features of individuals with DEE-SWAS and EE-SWAS. Brain-specific gene co-expression analysis was performed for D/EE-SWAS genes.
Results: We identified the etiology in 42/91 (46%) patients in our Core cohort, including 29/44 (66%) with DEE-SWAS and 13/47 (28%) with EE-SWAS. A genetic etiology was identified in 31/91 (34%). D/EE-SWAS genes were highly co-expressed in brain, highlighting the importance of channelopathies and transcriptional regulators. Structural etiologies were found in 12/91 (13%) individuals. We identified 10 novel D/EE-SWAS genes with a range of functions: *ATP1A2*, *CACNA1A*, *FOXP1*, *GRIN1*, *KCNMA1*, *KCNQ3*, *PPFIA3*, *PUF60*, *SETD1B, and ZBTB18*, and 2 novel copy number variants, 17p11.2 duplication and 5q22 deletion. Although developmental regression patterns were similar in both syndromes, DEE-SWAS was associated with a longer duration of epilepsy and poorer intellectual outcome than EE-SWAS.
Interpretation: DEE-SWAS and EE-SWAS have highly heterogeneous genetic and structural etiologies. Phenotypic analysis highlights valuable clinical differences between DEE-SWAS and EE-SWAS which inform clinical care and prognostic counseling. Our etiological findings pave the way for the development of precision therapies.

ANN NEUROL 2024;00:1–12

Developmental and epileptic encephalopathy with spike–wave activation in sleep (DEE-SWAS) and epileptic encephalopathy with spike–wave activation in sleep (EE-SWAS) are rare childhood epilepsy syndromes with overlapping features and have been distinguished in the recent International League Against Epilepsy (ILAE) classification of epilepsy syndromes (see below).[1] They are characterized by regression or plateauing in development, which can affect speech and language, cognitive, behavioral, and motor domains. Seizures occur in some, but not all, individuals. These disorders are characterized by their remarkable electroencephalogram (EEG) signature of sleep activated, 1-2 Hz spike–wave activity in non-rapid eye movement (REM) sleep. Developmental sequelae often persist after remission of seizures and SWAS.[2]

In the 2022 ILAE epilepsy syndrome classification, DEE-SWAS is distinguished from EE-SWAS by pre-existing developmental impairment prior to onset of SWAS, whereas, in EE-SWAS, development is normal, and regression or plateauing occur with the evolution of SWAS.[1] The terms DEE-SWAS and EE-SWAS replace epilepsy syndromes that lie within the epilepsy-aphasia spectrum (EAS): atypical benign focal epilepsy of childhood (ABFE), encephalopathy with continuous spike–wave in sleep (E-CSWS), and Landau–Kleffner syndrome (LKS). In the 2022 ILAE epilepsy syndrome classification, LKS was retained as a specific syndrome within EE-SWAS.[1]

DEE-SWAS and EE-SWAS have heterogeneous etiologies. Genetic etiologies have emerged as the most important cause with a range of copy number variants (CNVs) and single genes implicated. *GRIN2A*, encoding the alpha-2 NMDA glutamate receptor subunit, accounts for 9–20% of cases of patients with D/EE-SWAS, usually associated with loss of channel function.[3–5] *CNKSR2*, encoding a synaptic scaffolding protein, is an X-linked recessive cause.[6] Structural causes include malformations of cortical development, such as polymicrogyria, thalamic insults,[7,8] and hydrocephalus with a ventriculoperitoneal shunt.[9]

Here, we sought to understand whether the DEE-SWAS from EE-SWAS have different phenotypic patterns, and to analyze the etiologies of DEE-SWAS and EE-SWAS in a large international cohort of patients. We expand the genetic landscape underpinning DEE-SWAS and EE-SWAS.

Methods

The Epilepsy Genetics research database at the University of Melbourne, Austin Health, was searched for patients who had DEE-SWAS or EE-SWAS (Core cohort: n = 91). In addition, 10 etiologically solved patients referred by collaborators were included in the Expanded cohort (total n = 101) and analyzed with regard to their epileptology and etiology. Inclusion criteria were:

1. Regression or slowing of speech and language, cognitive, behavioral, or motor skills.
2. Marked activation of spike–wave activity in non-REM sleep.

Following the ILAE definitions, we have defined DEE-SWAS as the presence of pre-existing developmental delay prior to the development of SWAS compared with normal development in those with EE-SWAS.[1]

Seizure types were classified according to the ILAE 2017 seizure classification.[10] The following data were obtained: age of seizure onset and offset, developmental course and regression, examination, EEG, and neuroimaging findings. Past medical and family history was obtained. Medical records were reviewed, including neuropsychological assessment where available.

A range of genetic testing methodologies was used: targeted sequencing using molecular inversion probes (MIPs) as previously described,[3] epilepsy gene panel,

exome or genome sequencing (singleton, trio, or Expanded family), chromosomal microarray, classical karyotype, and single gene sequencing, using clinical or research platforms. Variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines.[11]

Statistical analyses were performed using R version 4.0.5. Chi-squared and Fisher exact tests were used to assess correlations between DEE-SWAS and EE-SWAS with sex, seizures, intellectual disability, and etiology. The probability of seizure and SWAS remission were estimated using the Kaplan–Meier method with the survminer R package.[12] Here, the log-rank sum test was used to determine distribution differences between DEE-SWAS and EE-SWAS.

For gene co-expression analyses, normalized brain expression values from the BrainSpan Developmental transcriptome dataset were downloaded from http://www.brainspan.org. Genes were removed if they had expression values missing from >50% of the 524 samples available from 42 individuals as previously described.[13] Using the log2 transformed expression values, a matrix of weighted correlations was generated, with weights determined as $1/\sqrt{n}$, where n is the number of samples contributed by the respective individual. Correlation plots were visualized using the corrplot R package, with genes ordered by hierarchical clustering, using the median linkage method. To determine whether the identified DEE-SWAS/EE-SWAS gene clusters were more highly co-expressed than expected by chance, we randomly sampled 5,000 sets of genes of the same cluster size. We calculated the median $|\rho|$ for each random gene set and compared this to the observed median $|\rho|$ of the DEE-SWAS/EE-SWAS gene clusters.

Written informed consent was obtained from parents or legal guardians of minors or those with intellectual disability. This study was approved by the Human Research Ethics Committee of Austin Health (H2007/02961) and the institutional review boards of collaborating groups.

Results

The Epilepsy Research database contained 114 probands who had been noted to have DEE-SWAS or EE-SWAS, of whom 91 met the inclusion criteria and comprised the Core cohort. A total of 23 patients were excluded for the following reasons: 17 had inadequate information, 4 did not meet inclusion criteria, and 2 had epilepsy with myoclonic-atonic seizures (EMAtS) after review. An additional 10 etiologically-solved patients referred by collaborators were then included in the Expanded cohort, (n = 101).

We analyzed demographic data, epileptology, developmental trajectory, and neuropsychological profiles in the Expanded cohort (n = 101) and compared the clinical features of DEE-SWAS and EE-SWAS. We then confined our comparison of etiological findings between DEE-SWAS and EE-SWAS to the Core cohort (n = 91) as more meaningful conclusions could be drawn from an unselected case series. Previous epilepsy syndrome diagnoses were only known in the Core cohort.

The cohort of 101 individuals had a median age of 17.8 years (range 5–33 years); 54 (53%) were male. One patient died at age 12 years.

Epileptology

Of 101 patients, 93 (92%) patients had seizures with mean onset of 4 years (range 5 days- 11.3 years). Seizure offset, defined as a minimum of 2 years of seizure-freedom, occurred in 58 (62%) individuals, with a mean duration of epilepsy of 5.6 years.

Seizures at onset were focal (57/93, 61%), generalised (35/93, 38%) or unknown (1/93, 1%). With time, seizure types included both focal and generalized in 55/93, focal alone in 22/93 and generalized alone in 16/93 (Fig 1). 9 had convulsive status epilepticus (defined as a seizure lasting at least 30 minutes) and 16 had non-convulsive status epilepticus, with 3 patients having both.

All patients had at least a single sleep EEG showing SWAS. Serial EEGs were performed in 96/101 (95%), with an average of 6 recordings per person (508 routine EEGs, 38 24-hour ambulatory EEGs, 60 inpatient video-EEG monitoring). Average age of SWAS onset occurred at 6.3 years, 28 months after seizure onset. The offset of SWAS was known in 47 individuals, with SWAS lasting an average of 3.8 years (range 1 month—13.3 years) from initial detection. SWAS was ongoing in 14, with an average duration of 5.3 years, and SWAS outcome was unknown in 37 patients. In 3 patients, their epilepsy syndrome evolved to Lennox–Gastaut syndrome (LGS).

Eight patients had SWAS without seizures, including 5 with DEE-SWAS and 3 with EE-SWAS. Using the previous nomenclature, 2 had LKS, 4 EAS and 2 had E-CSWS.

Developmental Trajectory

Heterogeneous patterns of developmental regression and plateauing were observed, across speech and language, cognitive, behavioral, and motor domains (Fig 2A). While all 101 patients had regression or plateauing in at least 1 domain, 14 had regression across all domains. The most commonly affected domain was speech and language (78/101, 77%), followed by cognition (61/101, 60%).

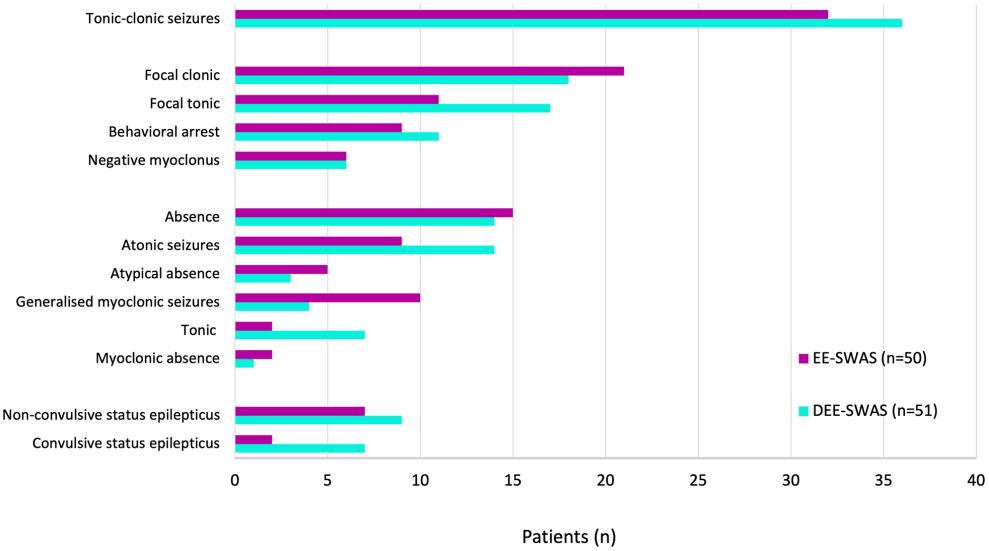

FIGURE 1: Seizure types in DEE-SWAS and EE-SWAS.

Behavioral regression occurred in 50/101, of whom, 13 did not have speech or language regression.

Neuropsychological Assessment

Neuropsychological assessment was performed in 72/101 (71%) patients, with the remaining 29 patients having the severity of intellectual disability estimated based on their daily functioning. Intellect varied from normal intellect in 17/101 (17%), to mild intellectual disability in 55 (54%), moderate in 17 (17%), severe in 10 (10%) and profound impairment in 2 (2%) (Fig 2B).

Epilepsy Syndromes: DEE-SWAS Versus EE-SWAS

Our cohort comprised 51/101 (50%) individuals (31 males, 61%) with DEE-SWAS, and 50/101 (50%) individuals with EE-SWAS (23 males, 46%).

Seizures occurred in similar proportions of patients with DEE-SWAS (46/51, 90%) and EE-SWAS (47/50, 94%) (Table), with earlier mean seizure onset in DEE-SWAS (3.3 years) compared with EE-SWAS (4.4 years). Patients with DEE-SWAS had seizures for longer (median 10.0 years) compared with those who had EE-SWAS (median 5.2 years) ($p = 0.00013$, log-rank test) (Fig 3A). Correspondingly, SWAS was present for longer in patients with DEE-SWAS (median 8.9 years) compared with those who had EE-SWAS (median 5.8 years) ($p = 0.071$, log-rank test) (Fig 3B).

Patients with EE-SWAS and DEE-SWAS showed similar patterns of regression involving speech and language, cognition, motor skills, and behavior (Fig 2A). Intellectual outcome was better in patients with EE-SWAS (92% normal intellect or mild intellectual disability)

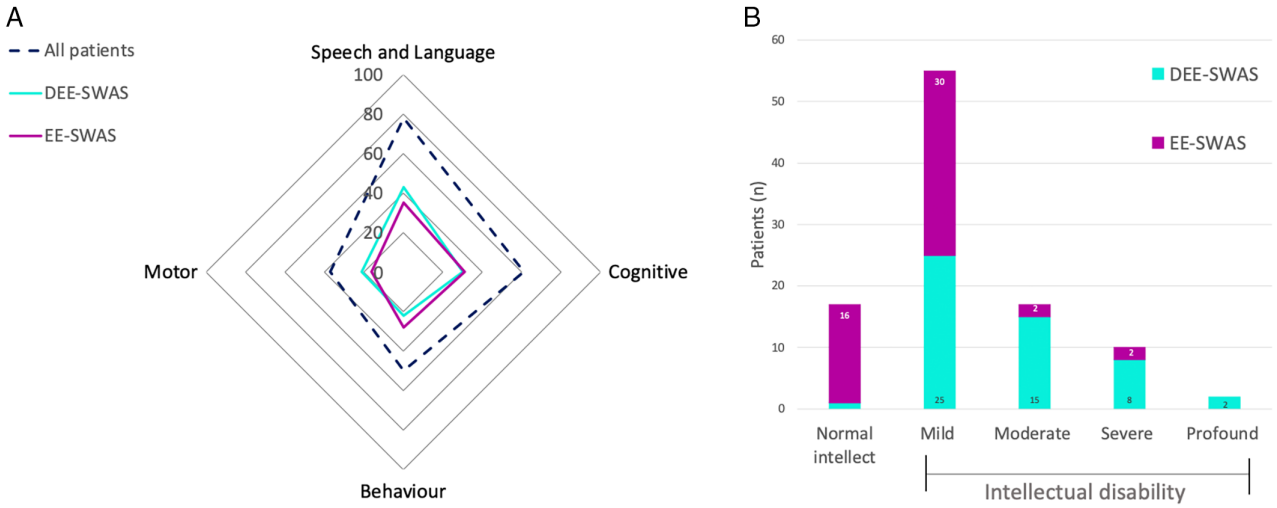

FIGURE 2: (A) Developmental regression by domain (speech and language, cognitive, behavior and motor) in individuals with DEE-SWAS and EE-SWAS. (B) Intellectual outcome in patients with DEE-SWAS and EE-SWAS.

TABLE. Clinical Features and Etiology of Individuals with DEE-SWAS and EE-SWAS

Expanded cohort, n = 101	DEE-SWAS n = 51 (%)	EE-SWAS n = 50 (%)	*p*-value[b]	Adjusted *p*-value[d]
Demographic data				
Male	31/51 (61%)	23/50 (46%)	0.20	1
Clinical features				
Seizures[c]	46/51 (90%)	47/50 (94%)	0.72	1
Intellectual disability (moderate to profound)[c]	25/51 (49%)	4/50 (8%)	$\mathbf{5.5 \times 10^{-6}}$	$\mathbf{6.1 \times 10^{-5}}$
Core cohort, n = 91	**DEE-SWAS, n = 44 (%)**	**EE-SWAS, n = 47 (%)**	***p*-value[b]**	**Adjusted *p*-value[d]**
Etiologically solved, 42/91	29/44 (66%)	13/47 (28%)	**0.0006**	**0.007**
Genetic etiology (31/91)[a]	24/44 (55%)	7/47 (15%)	**0.0002**	**0.002**
Single gene variants	17/44 (39%)	6/47 (13%)	0.009	0.10
Copy number and chromosomal abnormalities[c]	7/44 (16%)	1/47 (2%)	0.03	0.33
Structural etiology (12/91)[a]	5/44 (11%)	7/47 (15%)	0.85	1
Malformation of cortical development[c]	3/44 (7%)	2/47 (4%)	0.67	1
Unilateral thalamic lesion[c]	0	5/47 (11%)	0.06	0.66
Hydrocephalus with ventriculoperitoneal shunting[c]	2/44 (5%)	0	0.23	1

[a]1 patient with EE-SWAS had both a genetic etiology (*NPRL2* pathogenic variant) associated with a structural etiology (polymicrogyria).[17]
[b]*p*-values calculated using chi-square or Fisher's exact test (when the total number in one or more cells was <5)[c].
[d]Adjusted for the total of 11 tests performed using Bonferroni correction.

compared with those who had DEE-SWAS (49% moderate to profound intellectual disability) (Fig 2B, Table).

Previous epilepsy syndrome diagnoses in the Core cohort (n = 91) included 11 with ABFE, 48 E-CSWS, 10 LKS, and 22 unclassified EAS (Fig 4A). We observed an interesting evolution of DEE-SWAS to LGS in 3 patients. Tonic seizures only arose 3, 8, and 13.5 years after seizure onset. Initial EEGs did not show paroxysmal fast activity and background slowing; both features arose 3, 15, and 19.5 years after SWAS was identified. All have drug-resistant seizures with moderate to profound intellectual disability (Fig 4B).

Etiology

The etiology was identified in 42/91 (46%) patients in the Core cohort, including 29/44 (66%) with DEE-SWAS and 13/47 (28%) with EE-SWAS (Table). Genetic causes predominated with 31/91 (34%) patients having a genetic etiology and 12/91 (13%) a structural cause. Notably, this included 1 individual who had both a genetic (*NPRL2*) and structural (polymicrogyria) etiology (Fig 5). Of the 49/91 who remain unsolved, all had some genetic testing including genome sequencing in 7, exome sequencing in 27, epilepsy gene panel in 11, and chromosomal microarray in 4. Of the additional 10 patients included in the Expanded cohort, 9 had a genetic and 1 a structural etiology.

Genetic Findings

A pathogenic variant was identified in 40/101 unrelated patients in the Expanded cohort (31 pathogenic, 9 likely pathogenic by ACMG criteria)[11] (Fig 6). This included 20 single gene variants in 32 individuals (10 *GRIN2A*, 3 *CNKSR2*, 2 *SCN2A*). There were also 6 CNVs in 7 patients, and a chromosomal abnormality in 1 patient. A total of 15 solved cases have been previously published[3,6,14–22] (Table S1).

Pathogenic variants arose *de novo* in 24/40 individuals. A total of 12 patients had inherited variants from their mother (5) or father (7). In 4, the inheritance was unknown. For the 12 probands with an inherited variant, there were 28 family members who carried the pathogenic variant, with 26 being affected. This included 17 with *GRIN2A* (11 published[3,22]), 3 with *CNKSR2* (3 published[6]),

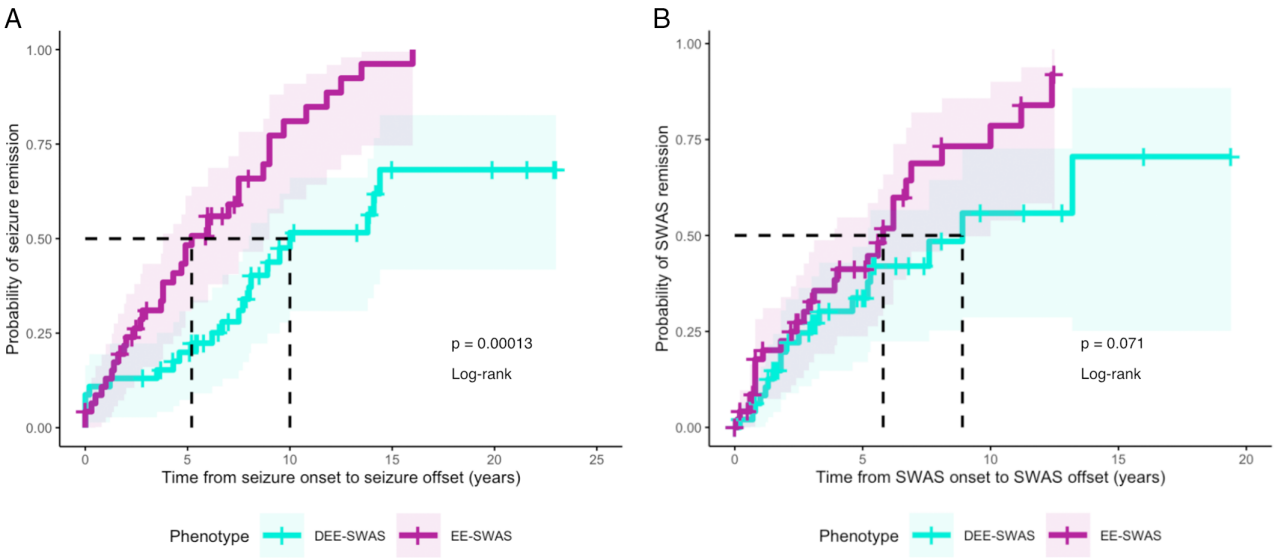

FIGURE 3: (A) Probability of time to seizure remission (n = 93). (B) Probability of time to SWAS remission (n = 101). Probability was calculated using the Kaplan–Meier method for survival analysis. The shaded color regions define the 95% confidence intervals. Colored vertical dashes along each survival curve denote the most recent age of individuals known to still be experiencing seizures (A) or SWAS (B) (censored observations). Median time to event (seizure or SWAS offset) for each group indicated by black dashed lines.

and 2 sets of monozygotic twins who shared their pathogenic variant (isodicentric(15;15) (q13:q13)) with both twins having DEE-SWAS; *SCN1A* with cotwin having febrile seizures with intellectual disability and autism spectrum disorder[20] (Table S1).

Nine patients had a recognized genetic syndrome. DEE-SWAS or EE-SWAS have not been previously linked to 5q22 deletion, *FOXP1-DEE*, Verheij syndrome (*PUF60*)[19] and Potocki-Lupski syndrome (17p11.2duplication). It has been previously described in 15q duplication,[23] 16p11.2 duplication,[21] Xp11.23-p11.22 microduplication,[24] Rett syndrome (*MECP2*)[25] (our patient died at age 12 years), and Coffin-Siris syndrome (*ARID1B*).[26]

Single Gene Variants

A total of 32 patients had pathogenic variants in 1 of 20 genes (Fig 6). Inheritance patterns varied with 17 genes following autosomal dominant, 2 X-linked recessive (*CNKSR2*, *CUL4B*) and 1 X-linked dominant (*MECP2*) inheritance. A total of 20 variants were missense, and 12 were protein truncating variants (4 frameshift, 5 nonsense, 2 splice site and 1 start loss). Pathogenic variants were found in *GRIN2A* (10 probands),[3,22] *CNKSR2* (3 probands),[6] and *SCN2A* (2 patients). Seventeen genes were implicated in a single patient.

We identified 10 novel DEE-SWAS and EE-SWAS genes: *ATP1A2*, *CACNA1A*, *FOXP1*, *GRIN1*, *KCNMA1*, *KCNQ3*, *PPFIA3*, *PUF60*, *SETD1B*, and *ZBTB18*; and 10 previously described DEE-SWAS and EE-SWAS genes: *ARID1B*,[26] *CNKSR2*,[6] *CUL4B*,[27] *GRIN2A*,[3–5,22] *GRIN2B*,[28] *KCNH5*,[16] *MECP2*,[25] *SCN1A*,[20] and *SCN2A*.[29] One patient with EE-SWAS had polymicrogyria due to a maternally inherited *NPRL2* variant.[17] Causative genes had a range of cellular functions:[30] ion channel subunits (*ATP1A2*, *CACNA1A*, *GRIN1*, *GRIN2A*, *GRIN2B*, *KCNH5*, *KCNMA1*, *KCNQ3*,[31] *SCN1A*, *SCN2A*), transcriptional regulation (*ARID1B*, *CUL4B*, *FOXP1*, *MECP2*, *PUF60*, *SETD1B*, *ZBTB18*), scaffolding (*CNKSR2*), mechanistic target of rapamycin [mTOR] pathway (*NPRL2*), and cell adhesion regulation (*PPFIA3*) (Fig 6).

A brain-specific gene co-expression analysis was performed to determine if the implicated genes were more commonly co-expressed than would be expected by chance to suggest an underlying shared biological mechanism. The ordered correlation matrices revealed two clusters of positively correlated gene sets (blue) that accounted for all DEE/EE-SWAS genes with the exception of *ATP1A2* and *NPRL2* (Fig 7). Cluster 1 included *GRIN2B*, *KCNH5*, *KCNQ3*, *CACNA1A*, *GRIN1*, *PPFIA3*, *SCN2A*, *CNKSR2*, *SCN1A*, *KCNMA1*, and *GRIN2A*, predominantly encoding ion channels. Cluster 2 principally captures genes involved in transcriptional regulation: *FOXP1*, *PUF60*, *CUL4B*, *MECP2*, *ARID1B*, *SETD1B*, and *ZBTB18*. Using a Monte Carlo sampling approach, we found evidence that the two DEE/EE-SWAS gene clusters were more highly co-expressed than would be expected by chance; cluster 1 ($p = 0.0002$), cluster 2 ($p = 0.04$). These results suggest that the cluster 1 genes, in particular, have similar brain gene expression signatures.

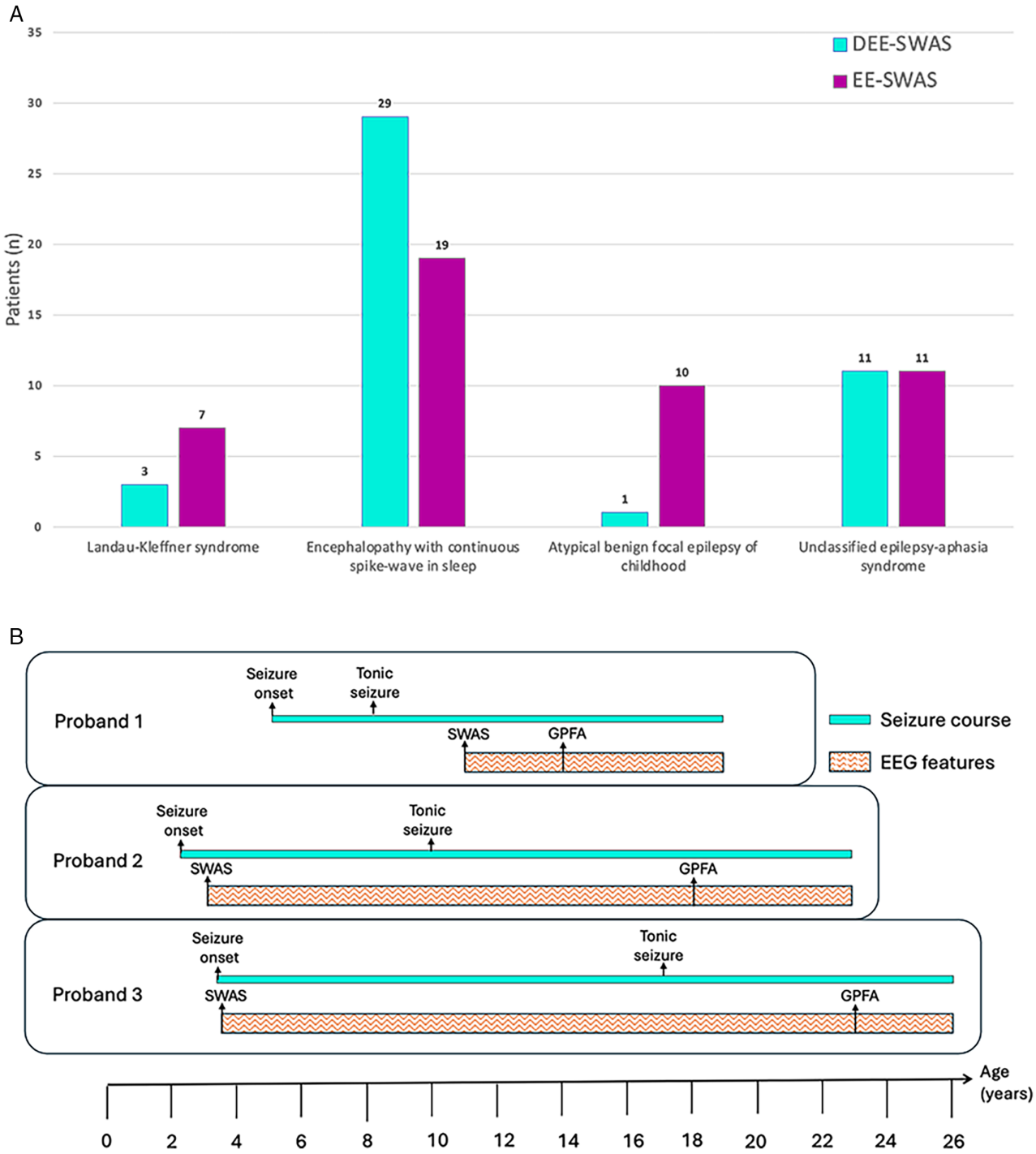

FIGURE 4: Epilepsy syndromic terminology and evolution. (A) Mapping of old ILAE terminology of epilepsy syndromes diagnosed in patients with the epilepsy-aphasia spectrum (Landau–Kleffner syndrome, encephalopathy with continuous spike–wave in sleep, atypical benign focal epilepsy of childhood, unclassified) to 2022 ILAE terminology of DEE-SWAS and EE-SWAS. (B) Evolution from DEE-SWAS to LGS in 3 probands. SWAS, spike–wave activation in sleep; GPFA, generalized paroxysmal fast activity.

Inter-connections between clusters are also supported by the observed negative correlations (red) between the two gene clusters (Fig 7).

Ten probands had *GRIN2A* pathogenic variants. There were 2 recurrent variants in 4 individuals (Table S1); the probands who shared the *GRIN2A* p.-Phe139Ilefs*15 variant were later shown to be related.[22] Two patients had *de novo* variants, while 5 variants were paternally and 2 maternally inherited (1 unknown). A total of 4 were protein truncating variants (1 nonsense, 1

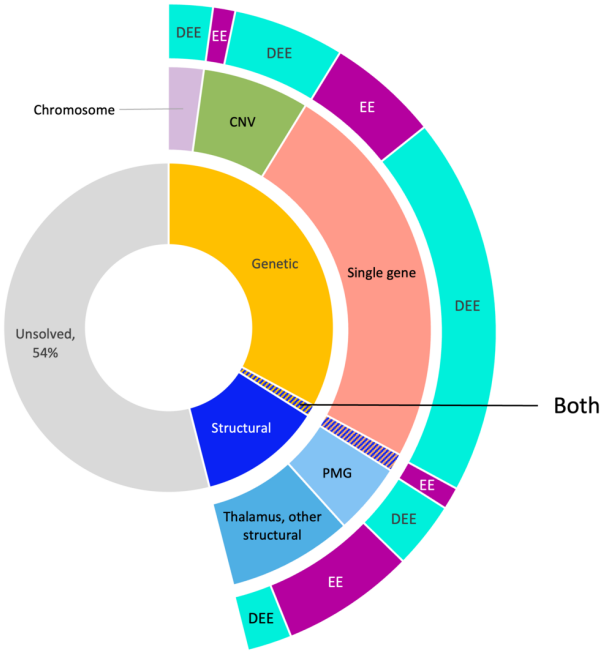

FIGURE 5: Solved cases in our Core cohort, n = 91, by etiology and epilepsy syndromes.

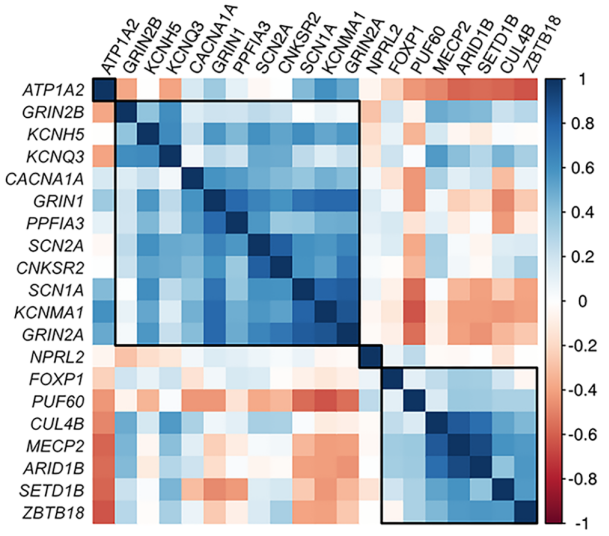

FIGURE 7: Gene co-expression matrix for 20 DEE-SWAS and EE-SWAS genes. Pairwise Spearman correlations between genes shown, based on 524 samples from 42 individuals from the BrainSpan resource.[13] Genes are ordered and grouped with hierarchical clustering, using the median linkage method. Black boxes denote clusters of highly correlated genes (top left box for cluster 1 and bottom right box for cluster 2).

start loss, and 2 splice donor mutation) and 6 missense. Two variants were novel (NM_001134407.3, p.Val522Gly, and p.Tyr393Ter).

Seven of the 10 probands had 17 affected family members. Of the total 27 individuals with *GRIN2A* pathogenic variants, epilepsy occurred in 25/27 (information unavailable in 1), with seizures beginning from 23 months to 10 years. Intellectual disability occurred in 21/27 individuals with 18 having mild, 2 moderate, and 1 severe intellectual disability; 6 were of normal intellect. Speech and language difficulties were found in all 24/27 for whom data were available. This included speech dyspraxia in 16/27 and speech and language regression in 10. 5/6 individuals of normal intellect had protein truncating variants. All 3 with moderate to severe intellectual disability had missense variants. For 3 variants (p.Asp731Asn, p.Thr531Met, p.Cys231Arg), functional studies have previously shown loss of channel function.[32]

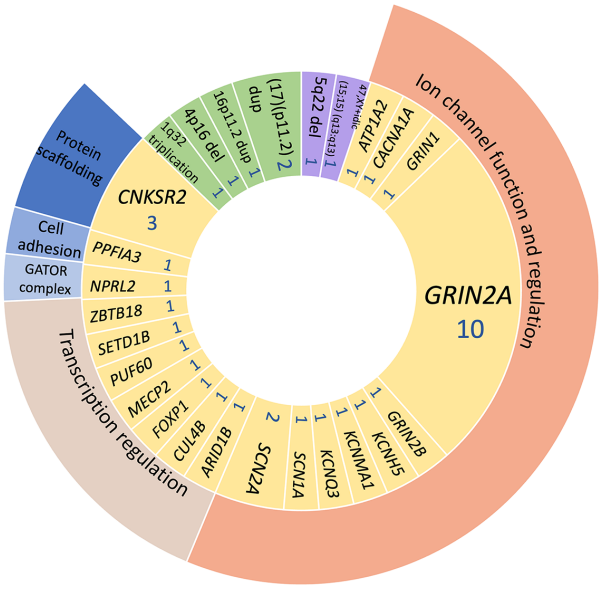

FIGURE 6: Genetic findings in patients with DEE-SWAS and EE-SWAS in the Expanded cohort highlighting functional roles.

CNVs and Chromosomal Abnormalities

CNVs were found in 7 patients, including 5 duplications and 2 deletions. There were 2 individuals with 17p11.2 duplication, which is associated with Potocki-Lupski syndrome when *RAI1* is duplicated.[33,34] RAI1 protein disrupts the circadian rhythm gene function which has been attributed to underlie the disrupted sleep patterns seen in patients with Potocki-Lupski syndrome.[34] Our female patient (Patient 39), with a 3.5 Mb duplication including *RAI*, had features consistent with Potocki-Lupski syndrome. Our male patient (Patient 38) had a 2.6 Mb duplication which did not include *RAI1* and did not have the phenotypic features of Potocki-Lupski syndrome;[33,34] this is therefore a new CNV causing DEE-SWAS (Fig 6). We did not find any other known epilepsy genes in this region.[35] DEE-SWAS has also not been previously reported in individuals with 5q22 deletion; our patient had a 14.5 Mb deletion including *APC* and *KCNN2*.

Previously described CNVs associated with DEE-SWAS and EE-SWAS in our series included 16p11.2 duplication,[21] 4p16 deletion,[21] 1q32 duplication,[21] and Xp11.23-p11.22 duplication.[24] DEE-SWAS is an established feature of Xp11.23-p11.22 duplication syndrome, as found in our female patient. Epilepsy genes in this duplicated region include *IQSEC2*, *CASK*, *ATP6AP2*, *SYN1*, *SMC1A*, *CCDC22*, *KDM5C*, *NDP*, and *SLC35A2*.[24]

A chromosomal abnormality was found in a monozygotic twin with isodicentric chromosome 15.[23]

MRI Findings

Of the 88 patients in the Core cohort who had at least 1 brain MRI, 72/88 (82%) MRIs were normal. Of the 16 patients with abnormal MRIs, 5 had malformations of cortical development, 7 had (likely) acquired lesions, and 4 had incidental findings (3 non-progressive developmental venous anomalies, 1 had minor bilateral peritrigonal white matter signal abnormalities without a history of hypoxic–ischemic encephalopathy).

The malformations of cortical development included 3 individuals with bilateral perisylvian polymicrogyria, and two with unilateral polymicrogyria (left frontal temporal in 1, and extensive right hemispheric frontal, temporal, parietal and perisylvian polymicrogyria in the other).

Of the 7 patients thought to have acquired lesions, 5 had unilateral, non-progressive (repeated studies over 2–6.5 years in 3 cases) thalamic lesions involving anterolateral, medial or posteromedial thalamic regions. None of these 5 patients had a history of perinatal seizures; however, in 2 cases, the thalamic lesions were attributed to post-hemorrhagic ischemia based on vascular imaging (3 T MRA, MRV) performed at ages 8 and 10 years respectively. One individual had perinatal right germinal matrix hemorrhage and the other had bilateral intraventricular hemorrhage with extra-axial extravasation. Both patients developed hydrocephalus requiring bilateral ventriculoperitoneal shunting.

EEG lateralization was concordant with MRI findings in 9/12 patients. All 4 patients with bilateral structural abnormalities had bilateral discharges, and 5/8 of those with unilateral abnormalities had ipsilateral discharges in wakefulness, and bilaterally synchronous discharges in sleep.

One patient from the Expanded cohort had EE-SWAS in the setting of a left-sided thalamic hemorrhage. The timing and cause of the hemorrhage was uncertain as this patient presented with seizures at 2 years 7 months and developed SWAS 21 months later.

Discussion

We analyzed the clinical and etiological features of a cohort of 101 individuals with the epilepsy syndromes of DEE-SWAS and EE-SWAS. We discovered the etiology in 46% of the Core cohort in individuals with DEE-SWAS, and EE-SWAS and found marked genetic and structural heterogeneity. We identified 20 genes, 6 CNVs, and 1 chromosomal abnormality in our cohort, implicating many neurobiological pathways including ion channels, transcriptional and synaptic regulation (Fig 6). Structural etiologies ranged from polymicrogyria, to unilateral thalamic lesions and post-hemorrhagic acquired hydrocephalus.

Is Distinguishing the Epilepsy Syndromes of DEE-SWAS and EE-SWAS Meaningful?

As DEE-SWAS and EE-SWAS were distinguished in the 2022 ILAE classification,[1] we asked whether this separation is clinically and etiologically meaningful. The classification states that seizures and the SWAS EEG signature in DEE-SWAS and EE-SWAS are expected to remit in adolescence.[1,2] We found that DEE-SWAS was associated with a younger age of seizure onset, longer duration of epilepsy, and less likelihood of seizure remission. Patients with EE-SWAS had a 50% probability of seizure remission after a 5 year history of seizures, compared with a 50% probability of seizure remission after 10 years for those with DEE-SWAS (Fig 3A). This reflects the longer duration of epileptic encephalopathy and associated poorer intellectual outcome in patients with DEE-SWAS compared to those with EE-SWAS. It is possible that more aggressive management may have the potential to ameliorate the long-term impact on cognition.

Our etiological yield was higher in patients with DEE-SWAS (29/44, 69%) than EE-SWAS (13/47, 28%; Table). Notably, 24/44 (55%) DEE-SWAS patients had an identified genetic etiology compared to only 7/47 (15%) EE-SWAS patients. Structural findings occurred at a similar frequency in DEE-SWAS (5/44, 11%) and EE-SWAS (7/47, 15%).

Genetic and Structural Etiologies

A genetic etiology was identified in 31/91 (34%) individuals in our Core cohort (Fig 5), with 10 additional cases with known etiologies added to the Expanded cohort (Fig 6). Only 1 individual had both a structural and genetic etiology, bilateral perisylvian polymicrogyria due to a known genetic etiology, *NPRL2*.[17] Of the Core cohort, genetic findings were identified in 24 DEE-SWAS and 7 EE-SWAS cases, with single gene abnormalities in 17 and 6, respectively. In the whole cohort of 101 patients, there were 20 genes and 8 chromosomal or CNVs identified (Fig 6). This brings the total number of genes implicated in the etiology of DEE-SWAS and EE-SWAS to 43 (Table S2).

GRIN2A pathogenic variants were the most common etiology in the Core cohort, accounting for 23% (7/31) of our genetically solved cases.[3,22] Phenotypic heterogeneity was common, both within families and for the same pathogenic variant across different families.[22,32] Ten individuals in the Expanded cohort had *GRIN2A* pathogenic variants, with all having epilepsy and intellectual disability, and 50% had speech dyspraxia. We had limited information on 17 relatives with 25/27 having a history of seizures, 21 intellectual disability, and 16 speech dyspraxia.

It has been hypothesized that pathogenic *GRIN2A* missense variants in transmembrane and linker domains are associated with more severe phenotypes than protein truncating variants and missense variants in other domains.[32] This is supported by our 6 individuals of normal intellect as 5/6 had protein truncating variants and the sixth had a proven loss-of-function missense variant (p.Thr531Met) in the S1 ligand binding domain.[32] However, the hypothesis does not hold true for 2 other families in our study. An unrelated proband in our cohort had the same p.Thr531Met variant yet had a severe phenotype, with moderate intellectual disability, indicating variant location and functional effect alone are not sufficient to explain the phenotype. Two siblings of another proband (Patient 15) had moderate and severe intellectual disability respectively; they shared the p.Val522Gly missense variant in the S1 domain, which is inconsistent with the suggested phenotype–genotype correlation.

7/10 *GRIN2A* pathogenic variants were inherited. Most affected probands and family members were of normal intellect or had mild intellectual disability. Understanding phenotypic pleiotropy is critical to ensure accurate reproductive counseling.

We have previously demonstrated that DEE genes co-express in the brain.[36,37] We therefore were interested in whether our functionally heterogeneous DEE-SWAS and EE-SWAS genes may also share brain co-expression signatures. The advantage of a brain gene co-expression-approach is that it is unbiased and simply analyzes whether genes have similar biological signatures. This analysis is thus not biased against genes with little functional data, an issue inherent in networks generated by alternate approaches, such as protein–protein interactions or text-mining. Indeed, our brain gene co-expression analysis determined that two subsets of DEE-SWAS and EE-SWAS genes significantly co-express in the brain representing 2 functional groups: channelopathies and transcriptional regulators. As our brain co-expression analysis was based on tissue samples taken from individuals of different ages, further insights may be gained by looking at brain co-expression data from individuals at the specific age range at which D/EE-SWAS occurs. This may be highlighting biologically relevant SWAS pathways that may inform future mechanistic research and potential therapeutic avenues.

MRI brain revealed an etiology in 12/91 (13%) patients in the Core cohort, and included malformations of cortical development (5), unilateral thalamic lesions (5), and post-hemorrhagic hydrocephalus (2); all are established causes of DEE-SWAS and EE-SWAS.[7–9] The thalamus plays an integral role in the development of SWAS as it is the main generator of sleep spindles and spike–wave activity. Disruption of the balance between excitatory cortical neurons and inhibitory thalamic neurons leads to spike–wave discharges.[38] Thalamic volume loss in patients with polymicrogyria is a proven risk factor for development of DEE-SWAS and EE-SWAS.[8] Ventriculoperitoneal shunting in infancy, which occurred in 2 of our patients, is also postulated to disrupt thalamocortical networks.[9] The thalamus is thus central to the SWAS network.

Apart from one patient with unilateral polymicrogyria who had an inherited *NPRL2* variant, no other patients with polymicrogyria had a known pathogenic variant. Brain tissue was not, however, available for testing, and somatic mosaicism commonly underpins polymicrogyria. Importantly, sequencing was not targeted towards polymicrogyria genes.[39]

Insights into Syndromology

We observed an interesting novel syndromic evolution from DEE-SWAS to LGS in 3 patients. Two had known etiologies: bilateral polymicrogyria with subependymal nodular heterotopia in one, and Coffin-Siris syndrome due to an *ARID1B* pathogenic variant in the other. Recognition of this syndromic evolution emphasizes the need for more cautious prognostic counseling and the need for long-term follow-up of patients with DEE-SWAS.

It can be challenging to differentiate the syndrome of epilepsy with myoclonic-atonic seizures (EMAtS) from EE-SWAS. We had 2 patients who were initially misclassified due to their EEG findings of SWAS and developmental regression; however, they had myoclonic-atonic seizures. All 3 syndromes, EMAtS, DEE-SWAS, and EE-SWAS, can have regression with spike–wave activation in sleep, but patients who have myoclonic-atonic seizures have the syndrome of EMAtS. Myoclonic-atonic seizures, the key distinguishing feature, should be highlighted in the ILAE epilepsy syndrome classification as an alert in DEE-SWAS and EE-SWAS directing the clinician to consider the syndrome of EMAtS which carries different therapeutic approaches.

LKS has been retained as a subset of EE-SWAS in the 2022 classification.[1] However, our cohort

demonstrated that patients with LKS can have either EE-SWAS or DEE-SWAS, similar to previous studies that emphasize that pre-existing speech delay is possible in LKS.[40,41] Our 10 patients with an initial diagnosis of LKS were reclassified: 3 had DEE-SWAS and 7 EE-SWAS. All 3 with DEE-SWAS had pre-existing speech delay and regressed at age 2–5 years.

Our study has several limitations. Even though our cohort is large for these rare epilepsy syndromes, each etiological group remains relatively small making it challenging to draw definitive conclusions. EEGs were not always performed contemporaneously with developmental regression, due to access to EEG testing and delayed referrals to specialist care especially in children without seizures. It was also difficult to delineate precise offset of SWAS unless regular EEG studies in sleep are performed. This adds to the need for a more streamlined approach in monitoring and management of patients with DEE-SWAS and EE-SWAS.

Conclusion

We report a large cohort of patients with DEE-SWAS and EE-SWAS and dissect the clinical and etiological differences between these syndromes. Distinguishing DEE-SWAS from EE-SWAS aids prognostic counseling regarding epilepsy duration and cognitive function, and likely yield of genetic testing. Where a pathogenic variant is identified, reproductive counseling and cascade testing in family members should be performed. Understanding the etiology is critical to move towards precision medicine approaches for these severe epilepsy syndromes.

Author Contributions

I.E.S. and H.C.M. conceived and designed the study. I.E.S., H.C.M., S.V., K.L.O., B.M.R., A.L.S., C.T.M., M.G.M., A.J.L., J.A., R.W., M.C., G.M.S., A.T.G.C., E.R., R.I.T., S.M., R.J.L., D.G., S.F.B., M.S.H., B.S.G., K.B.H., J.D.S., A.B., L.G.S., and S.M.Z. contributed to the acquisition and analysis of data. S.V., K.L.O., and I.E.S. drafted the manuscript and figures.

Acknowledgements

We thank the patients and their families for participating in our research. We thank Dr Matthew Edwards for referring a patient to the study.

This work was supported by funding from the National Health and Medical Research Council (GNT1091593, GNT1196637, GNT1172897, GNT2006841), NIH (R01NS069605), Cure Kids New Zealand, Health Research Council of New Zealand, and Murdoch Children's Research Institute. 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.

Potential Conflicts of Interest

Nothing to report.

Data Availability

The data from this study are available upon reasonable request, by academic researchers with appropriate ethical approval.

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