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Diagnostic yield and clinical predictors of whole-exome sequencing in Thai children with developmental and epileptic encephalopathy and pharmacoresistant epilepsy: A prospective cohort study

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Abstract

Developmental and epileptic encephalopathies (DEEs) often have a monogenic basis, but prospective data from Southeast Asia remain limited. We evaluated the diagnostic yield, variant spectrum, and clinical actionability of singleton whole-exome sequencing (WES) — applied as a deliberate first-line strategy in a resource-limited setting — in 117 Thai children younger than 18 years with drug-resistant epilepsy (DRE) and suspected DEE, prospectively enrolled at a tertiary pediatric center between July 2024 and January 2026. Variants were interpreted per American College of Medical Genetics and Genomics guidelines and predictors examined with univariate logistic regression. Pathogenic or likely pathogenic variants were identified in 63 patients (53.8%); ion channel genes predominated, led by *SCN1A*. Earlier seizure onset (< 12 months) was associated with higher yield (odds ratio 2.32; 95% CI 1.11–4.96; *P* = 0.028). Yield was lowest in children with subtle structural imaging abnormalities (11.1%), a clinically useful negative finding for triaging WES allocation. Findings were clinically actionable in 68.3% of diagnosed cases, and median delay from seizure onset to molecular diagnosis was 81 months (IQR 33–131). In this largest prospective Southeast Asian DEE cohort to date, singleton WES — used deliberately as a first-line LMIC strategy — achieved a high diagnostic yield with substantial clinical utility, supporting earlier integration into pediatric epilepsy care across Southeast Asia and similar low- and middle-income settings.

INTRODUCTION

Epilepsy is a common neurological disorder with marked clinical and etiologic heterogeneity. A high-burden subgroup includes children with early-onset epilepsy, developmental impairment, and drug-resistant epilepsy (DRE). Within this spectrum, developmental encephalopathy refers to non-progressive developmental impairment attributable to a static brain disorder with coexisting epilepsy. By contrast, developmental and epileptic encephalopathy (DEE) describes severe disorders in which both the underlying pathology and epileptic activity contribute to cognitive and behavioral deterioration.(McTague et al. 2016; Scheffer et al. 2017; Scheffer et al. 2025) The etiology of DEEs is frequently genetic, particularly in early-onset and syndromic presentations. DEEs typically present in infancy or early childhood, with key features that include difficult-to-control seizures, developmental delay, intellectual disability, and other neurobehavioral comorbidities.(Wirrell et al. 2015)

Early-onset epilepsy is frequently genetic, particularly when structural, metabolic, infectious, and immune causes have been excluded.(Rochtus et al. 2020; Tsang et al. 2019; Zhang et al. 2020) With broader use of next-generation sequencing, the genetic landscape of DEEs has become clearer, highlighting substantial genetic heterogeneity. Many implicated genes converge on ion-channel function and synaptic transmission; recurrently reported genes include *SCN1A*, *SCN2A*, *KCNQ2*, *STXBP1*, and *CDKL5*. (Bashiri et al. 2023; Çapan Ö et al. 2024; Kocaaga and Yimenicioglu 2023; Rochtus et al. 2020; Sunnetci-Akkoyunlu et al. 2025; Tsang et al. 2019; Vetri et al. 2024; Zhang et al. 2020; Zou et al. 2024) Across epilepsy cohorts, the diagnostic yield of next-generation sequencing varies by phenotype, cohort composition, and testing approach(Bashiri et al. 2023; Çapan Ö et al. 2024; Kocaaga and Yimenicioglu

2023; Rochtus et al. 2020; Sunnetci-Akkoyunlu et al. 2025; Tsang et al. 2019; Vetri et al. 2024; Zhang et al. 2020; Zou et al. 2024); in DEEs, reported yields are approximately 22% to 43%. In pediatric DRE, yields range from 12% to 50%,(Rochtus et al. 2020; Tsang et al. 2019; Zhang et al. 2020) while in broader pediatric epilepsy cohorts show diagnoses on exome-based testing in 27% to 33% of cases.(Bashiri et al. 2023; Çapan Ö et al. 2024; Kocaaga and Yimenicioglu 2023; Sunnetci-Akkoyunlu et al. 2025; Vetri et al. 2024; Zhang et al. 2020; Zou et al. 2024) Current evidence indicates a substantial monogenic basis for early-onset epilepsy and reinforces the role of early comprehensive genomic testing.(Bashiri et al. 2023; Çapan Ö et al. 2024; Kocaaga and Yimenicioglu 2023; Rochtus et al. 2020; Sunnetci-Akkoyunlu et al. 2025; Tsang et al. 2019; Vetri et al. 2024; Zhang et al. 2020; Zou et al. 2024)

Identifying a causative variant in pediatric epilepsy directly informs clinical management, including antiseizure medication selection and targeted therapies, while also strengthening prognostic assessment and recurrence-risk counseling. In practice, sequencing strategy affects both diagnostic performance and cost: trio whole-exome sequencing (WES) generally provides a higher diagnostic yield and improves variant interpretation through inheritance analysis, often reducing variants of uncertain significance (VUSs), whereas singleton WES is more feasible as a first-line approach in low- and middle-income countries.

Despite these advances, data from Southeast Asia remain limited. To date, only a small number of regional studies have examined the genomic landscape of pediatric epilepsy, most with modest sample sizes, heterogeneous phenotypic inclusion, or reliance on gene-panel-based approaches, precluding direct comparison with WES-based cohorts reported elsewhere.(Hieu et al. 2022; Suwannachote et al. 2024) Diagnostic yield varies across studies according to phenotype, inclusion criteria, analytic methods, and ongoing gene discovery. We therefore conducted what is, to our knowledge, the largest prospective Southeast Asian cohort study of singleton WES in children with DRE and suspected DEE. We evaluated singleton WES not as a compromise but as a deliberate first-line strategy for resource-limited settings, in which trio testing carries an approximately three-fold higher per-case cost at our center. These findings inform implementation of genomic medicine in pediatric epilepsy care across Southeast Asia and other low- and middle-income settings.

MATERIALS AND METHODS

Study design and setting

This prospective cohort study was conducted at the Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, a tertiary pediatric referral center. Patients were enrolled from July 2024 to January 2026. The objective was to evaluate the diagnostic yield of singleton WES in children with DEE and DRE.

Ethics approval

statement and sample collection

The study was approved by the Siriraj Institutional Review Board (protocol number 513/2567[IRB1]; approval number Si-625/2024). All procedures adhered to the Declaration of Helsinki and the International Conference on Harmonization–Good Clinical Practice guidelines.

Informed consent

for the collection of 3–6 mL of peripheral blood was obtained from each participant and their parents. Written informed consent for publication was also secured.

Patient selection

Children were consecutively screened from the Neurology and Epilepsy Clinic. In addition, eligible patients were invited to participate through announcements made via the Child Neurology Association of Thailand during the study period. Eligibility was determined through review of clinical records and confirmation of predefined diagnostic criteria.

Inclusion criteria

Participants were eligible if they were < 18 years of age at enrollment, under ongoing care at Siriraj Hospital, and met at least one of the following clinical criteria:

- DEE, defined as epilepsy with developmental impairment (developmental delay or regression), in which epileptic activity contributes to developmental dysfunction, with supportive evidence from clinical features and/or epileptiform abnormalities on electroencephalography (EEG; eg, multifocal, generalized, or continuous epileptiform discharges, with or without background slowing).(Scheffer et al. 2017; Scheffer et al. 2025)
- DRE (defined as seizures persisting despite adequate trials of at least 2 appropriately chosen antiseizure medications(Kwan et al. 2010) used for a minimum of 6 months), together with developmental delay and intellectual disability.
- Diagnosis of a recognizable genetic epilepsy syndrome (eg, Dravet, Rett, or Angelman) without prior molecular confirmation.
- DRE with subtle or nonspecific structural abnormalities on neuroimaging (eg, mild cortical asymmetry, slight blurring of the gray–white matter junction, or nonspecific signal changes) insufficient to establish a definite structural etiology.

Exclusion criteria

Patients were excluded if a clear etiology of epilepsy had already been established, including inborn errors of metabolism, congenital structural brain malformations, prior brain insult with a defined cause (eg, traumatic brain injury, intracerebral hemorrhage, perinatal hypoxic-ischemic encephalopathy, delivery-related complications, or central nervous system infection with sequelae), or previously confirmed genetic disorders with an established molecular diagnosis.

Clinical data collection

Clinical data were obtained through structured review of medical records using a standardized case record form and managed with REDCap (Research Electronic Data Capture). This secure, web-based application is designed for research data collection and is hosted by the Research Data Management Unit, Faculty of Medicine Siriraj Hospital, Mahidol University. Collected variables included demographics, age at seizure onset, seizure history, developmental and comorbidity profiles, prior investigations, treatment history, and previous genetic testing.

The clinical utility of WES was categorized as follows: (1) change in current treatment using therapies available in Thailand; (2) identification of an approved genotype-directed therapy not currently available in Thailand; (3) surveillance for associated comorbidities; (4) prognostic counseling based on established genotype–phenotype correlations; and (5) family planning, defined as actionable reproductive counseling used in real-life decision-making. Data completeness was verified prior to analysis to ensure consistency and minimize missing variables.

WES and variant analysis

Peripheral blood samples (3–6 mL) were collected from patients with DRE with intellectual disability and DEE. Genomic DNA was extracted using the Gentra Puregene Blood Kit (Qiagen, Hilden, Germany). DNA concentration and purity were assessed using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

WES was performed by Macrogen Inc (Seoul, Republic of Korea) using the Illumina NovaSeq platform (Illumina Inc, San Diego, CA, USA), generating 150-base-pair paired-end reads (approximately 6 Gb per sample). Sequencing reads were aligned to the human reference genome (GRCh37/hg19) using the Burrows–Wheeler Aligner.(Li and Durbin 2009) Downstream processing and variant calling were conducted according to the Genome Analysis Toolkit (GATK, version 4.5) Best Practices workflow.(Van der Auwera et al. 2013) Mean sequencing depth across target regions ranged from 50X to 80X. Genomic variant call format (gVCF) files and final variant call format (VCF) files were generated independently for each sample.

Variant prioritization was performed to identify clinically relevant variants. Variants with low read depth, uncertain zygosity, or suspected pathogenicity were validated by Sanger sequencing. Parental Sanger confirmation was performed when relevant to variant interpretation.

Variant interpretation was performed using VCF files in accordance with the American College of Medical Genetics and Genomics guidelines,(Richards et al. 2015) implemented through the Franklin bioinformatics platform (Franklin by Genoox).(Genoox 2026) When applicable, disease-specific variant interpretation criteria were also applied. All candidate variants underwent assessment by clinical geneticists, neurologists, and variant scientists. Variants were classified into 5 categories: pathogenic, likely pathogenic, VUS, likely benign, and benign.

Variant rarity was assessed based on disease prevalence and allele frequencies from population databases, including the 1000 Genomes Project and the Genome Aggregation Database (gnomAD v2.1.1; hg19 alignment). Computational prediction tools included AlphaMissense for pathogenicity estimation,(Cheng et al. 2023; Yazaki et al. 2025) REVEL for missense variant prediction,(Hopkins et al. 2023; Ioannidis et al. 2016) and SpliceAI for evaluation of potential splicing effects.(Jaganathan et al. 2019; Walker et al. 2023) An epilepsy-associated gene list comprising 839 genes was used for gene–disease annotation, together with additional human phenotype ontology terms.(Gargano et al. 2024; Martin et al. 2019)

For downstream analysis, variants were grouped into 3 categories: positive (pathogenic or likely pathogenic); VUS; and negative (likely benign or benign). Among WES-positive cases, clinical actionability was classified as any of the following: changes in current treatment (limited to therapies available in Thailand), surveillance for comorbidities, prognostic counseling, and family planning.

Statistical analysis

Descriptive statistics were used to summarize baseline characteristics. Continuous variables are reported as median (IQR), and categorical variables as counts and percentages. The primary outcome was diagnostic yield, defined as the proportion of patients with a pathogenic or likely pathogenic variant divided by the total number of patients who underwent singleton WES.

Associations between clinical predictors and diagnostic yield were assessed using univariable logistic regression and are reported as odds ratios (ORs) with 95% CIs and *P* values. Predictors included prior clinical diagnostic subgroup, age at seizure onset, seizure type, selected phenotypic features, physical examination features, and EEG patterns. Age at seizure onset was analyzed both as a continuous variable to generate predicted probability curves and as a dichotomous variable (< 12 versus ≥ 12 months) in a separate model. Bubble plots were used to visualize subgroup prevalence and diagnostic yield, with bubble size proportional to subgroup size and shading indicating the direction and statistical significance of associations relative to the overall yield. All statistical tests were 2-sided, and $P < 0.05$ was considered statistically significant. Analyses and data visualization were performed using R, version 4.5.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

A total of 117 children with DEE and DRE without a confirmed molecular diagnosis underwent singleton WES. The median age at enrollment was 102 months (IQR, 56–160), and the median age at seizure onset was 7 months (IQR, 3–24). Males comprised 56.4% of the cohort. Seizure onset occurred most frequently during infancy (45.3%), followed by childhood (43.6%) and the neonatal period (11.1%). Baseline clinical characteristics, seizure features, comorbidities, and physical examination findings are summarized in Table 1.

Table 1
Baseline demographic and clinical characteristics of the study cohort

Clinical characteristics	**All patients (*N* = 117)**
Male, *n* (%)	66 (56.4)
Age at enrollment, mo, median (IQR)	102 (56–160)
Age at seizure onset, mo, median (IQR)	7 (3–24)
Age group, *n* (%)	
Neonatal	13 (11.1)
Infancy	53 (45.3)
Childhood	51 (43.6)
Family history of, *n* (%)	
Epilepsy	7 (6.0)
GDD	4 (3.4)
Seizure features, *n* (%)	
Focal	59 (50.4)
GTC	45 (38.5)
GT	45 (38.5)
Myoclonic	28 (23.9)
Epileptic spasms	23 (19.7)
Atonic	13 (11.1)
Absence	9 (7.7)
GC	4 (3.4)
Myoclonic atonic	2 (1.7)
Multiple seizure type	72 (61.5)
History of SE	30 (25.6)
DRE	107 (91.5)
Neurodevelopmental and psychiatric comorbidities, *n* (%)	

Values are *n* (%) unless otherwise specified. Percentages are of the full cohort (*N* = 117) except for the “Severity of GDD/ID” sub-rows, which are of the GDD/ID subgroup (*n* = 108). Median (IQR) is reported for continuous age variables. Patients may have multiple seizure types or comorbidities, so columns within seizure-features and comorbidity sections do not sum to 100%.

Clinical characteristics	All patients (*N* = 117)
GDD/ID	112 (95.7)
Severity of GDD/ID (denominator *n* = 112)	
Mild	8 (7.1)
Moderate	11 (9.8)
Severe	42 (37.5)
Unknown	51 (45.5)
Autism	20 (17.1)
ADHD	14 (12.0)
Developmental regression	31 (26.5)
Physical examination findings, *n* (%)	
Non-ambulatory	34 (29.1)
Microcephaly	32 (27.4)
FTT	21 (17.9)
Abnormal movement	19 (16.2)
Dysmorphic features	11 (9.4)
Ataxia	9 (7.7)

Values are *n* (%) unless otherwise specified. Percentages are of the full cohort (*N* = 117) except for the "Severity of GDD/ID" sub-rows, which are of the GDD/ID subgroup (*n* = 108). Median (IQR) is reported for continuous age variables. Patients may have multiple seizure types or comorbidities, so columns within seizure-features and comorbidity sections do not sum to 100%.

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; DRE, drug-resistant epilepsy; FTT, failure to thrive; GC, generalized clonic seizure; GDD, global developmental delay; GT, generalized tonic seizure; GTC, generalized tonic-clonic seizure; ID, intellectual disability; IQR, interquartile range; mo, months; SE, status epilepticus.

Diagnostic yield and clinical actionabilities

Pathogenic or likely pathogenic variants were identified in 63 of 117 patients, yielding an overall diagnostic rate of 53.8%. Diagnostic yield varied across 4 phenotypic subgroups:

- developmental and epileptic encephalopathy (38/70; 54.3%);
- intractable seizures with intellectual disability (22/34; 64.7%);

- intractable seizures with possible symptomatic epilepsy based on subtle structural abnormalities without a documented acquired event (1/9; 11.1%); and
- clinically recognized genetic epilepsy syndromes without prior molecular confirmation (2/4; 50.0%).

A total of 43 VUSs and 55 positive variants were identified at initial assessment. Following detailed phenotypic review and parental testing, 18.6% of VUSs (8/43) were reclassified as pathogenic or likely pathogenic, while the remainder remained classified as VUS or negative.

Among the 63 genetically diagnosed patients, WES findings were clinically actionable in 43 (69.7%). Among genetically diagnosed patients:

- 34.1% had modifications to currently available treatments in Thailand;
- 15.9% were eligible for newly approved therapies that were not yet available in Thailand;
- 34.1% required enhanced surveillance for comorbidities;
- 90.9% received genotype-informed prognostic counseling; and
- 18.2% obtained actionable reproductive counseling for family planning.

The diagnostic yields across clinical subgroups, VUS resolution, and clinical actionability are shown in Fig. 1.

Genetic landscape

To further delineate the molecular landscape underlying the diagnostic yield, the distribution of implicated genes by gene ontology categories is shown in Fig. 2. Ion channel genes predominate, largely driven by sodium and potassium channel disorders, including *SCN1A* (*n* = 9), *KCNQ2* (*n* = 3), *SCN2A* (*n* = 2), and *KCNA2* (*n* = 2). Chloride channel genes (*CLCN2*, *CLCN4*) and ATPase/transporter genes (*ATP1A2*, *ATP1A3*, *ATP7A*) were each identified in 1 patient.

Outside the channelopathy group, recurrent findings included *MECP2* within transcriptional regulation (*n* = 3), *STXBP1* within synaptic function (*n* = 3), and *UBA5* within protein homeostasis (*n* = 3), while metabolic genes were less common, with *GLUL* identified in 2 patients. Most remaining genes were observed in single individuals across developmental, structural, and signaling pathways (eg, *PCDH19*, *UBE3A*, *FGF12*), highlighting marked molecular heterogeneity. All pathogenic or likely pathogenic variants and corresponding clinical profiles are provided in **Online Resource 1**.

Factors influencing diagnostic yield

The relationship between prior clinical diagnostic subgroup and genetic diagnostic yield is shown in Fig. 3. Across subgroups, the highest diagnostic yield was observed in patients with intractable seizures and intellectual disability, followed by those with DEE, whereas the clinically recognized genetic epilepsy syndrome subgroup did not show a statistically significant association. In contrast, intractable seizures

with possible symptomatic epilepsy were associated with a lower diagnostic yield (11.1%; OR, 0.09; 95% CI, 0.00–0.53; *P* = 0.027).

Logistic regression was used to model diagnostic yield across age at seizure onset, and the predicted probability curve is shown in Fig. 4. Diagnostic yield was higher in patients with seizure onset within the first year of life (neonatal 64.3%; infancy 62.3%) compared with childhood onset (42.0%). In a separate binary model, seizure onset before 12 months was associated with higher odds of a genetic diagnosis compared with onset at ≥ 12 months (OR, 2.32; 95% CI, 1.11–4.96; *P* = 0.028).

Diagnostic yield across seizure types, clinical and phenotypic features, and EEG patterns using univariable logistic regression is shown in Fig. 5. Among the evaluated predictors, epileptic spasms were the only factor significantly associated with a lower likelihood of a positive genetic diagnosis, whereas other seizure types, phenotype and examination features, and EEG patterns did not show statistically significant associations.

DISCUSSION

In this prospective cohort of 117 Thai children with DRE and suspected DEE, singleton WES identified pathogenic or likely pathogenic variants in 53.8% of cases. This represents one of the largest prospective single-center cohorts of DEE in Thailand and Southeast Asia.

Earlier studies conducted before 2020 reported diagnostic yields between approximately 12% and 40% in pediatric epilepsy cohorts, depending on phenotype selection and sequencing strategy.(Rochtus et al. 2020; Tsang et al. 2019; Zhang et al. 2020) For example, Tsang et al reported a 12% yield in DRE using singleton WES,4 while other exome-based cohorts demonstrated yields of approximately 30% to 40%. (Rochtus et al. 2020; Zhang et al. 2020) In contrast, more recent studies after 2021, particularly those enriched for early-onset DEE, have reported higher yields ranging from 40% to 55% and up to 64% in a single study of infantile-onset drug-resistant epilepsy.(Bashiri et al. 2023; Boonsimma et al. 2023; Çapan Ö et al. 2024; Khusiwilai et al. 2024; Kocaaga and Yimenicioglu 2023; Sunnetci-Akkoyunlu et al. 2025; Suwannachote et al. 2024; Vetri et al. 2024; Zou et al. 2024) This increase in yield over time likely reflects ongoing epilepsy gene discovery, expansion of population reference datasets, and refinement of variant interpretation frameworks, supported by multidisciplinary review involving neurologists, neurogenetics specialists, geneticists, and variant scientists.

Accordingly, we reassessed the diagnostic performance of singleton WES as a more practical and less expensive alternative to trio testing, using current genetic epilepsy knowledge and interpretation standards. Our observed diagnostic yield of 53.8% is consistent with the recent reports.(Bashiri et al. 2023; Boonsimma et al. 2023; Çapan Ö et al. 2024; Khusiwilai et al. 2024; Kocaaga and Yimenicioglu 2023; Sunnetci-Akkoyunlu et al. 2025; Suwannachote et al. 2024; Vetri et al. 2024; Zou et al. 2024)

VUSs are more frequently encountered with singleton WES than with trio testing and often require systematic resolution. In our cohort, multidisciplinary review including detailed phenotype–genotype

correlation, targeted literature appraisal, and parental segregation analysis enabled reclassification of 18.6% of initially reported VUSs as pathogenic or likely pathogenic variants. Consistent with prior studies of dynamic exome reinterpretation, iterative reanalysis incorporating segregation data and complementary clinical investigations can meaningfully increase the diagnostic yield.(Rochtus et al. 2020) This approach facilitates translation of uncertain findings into clinically interpretable results and maximizes the clinical utility of singleton WES.

With respect to gene distribution, ion channel–related genes predominated, consistent with prior DEE cohorts.(Bashiri et al. 2023; Boonsimma et al. 2023; Çapan Ö et al. 2024; Kocaaga and Yimenicioglu 2023; Sunnetci-Akkoyunlu et al. 2025; Suwannachote et al. 2024; Vetri et al. 2024; Zou et al. 2024) *SCN1A* was the most frequently identified gene in our cohort, followed by *KCNQ2*, *SCN2A*, and others. Beyond channelopathies, implicated genes encompassed transcriptional regulation, synaptic transmission, protein homeostasis, and metabolic pathways, underscoring the marked molecular heterogeneity characteristic of DEEs. However, gene distributions are strongly influenced by cohort ascertainment. In many DEE series, *SCN1A* accounts for approximately 10% to 20% of molecular diagnoses.(Boonsimma et al. 2023; Çapan Ö et al. 2024; Suwannachote et al. 2024; Vetri et al. 2024) We initially anticipated a lower contribution from *SCN1A*, as classical Dravet cases at our center are often evaluated using targeted single-gene testing and were captured in our prior Dravet cohorts.(Viravan et al. 2021) Nevertheless, *SCN1A* variants in our study were detected within broader phenotypic categories, including patients previously classified as having DEE or intractable epilepsy with intellectual disability, particularly during earlier periods when genetic testing was less accessible and Dravet syndrome was less well recognized.

Notably, *SCN1A* was also identified in a child initially suspected to have subtle focal cortical dysplasia who underwent lesionectomy without improvement. Following the genetic diagnosis, retrospective phenotyping supported a Dravet-spectrum disorder, and clinical management was modified accordingly. This observation underscores the importance of careful longitudinal history review, particularly in early-onset, intractable epilepsy. Genetic epilepsies may be misattributed to focal structural etiologies when EEG shows focal epileptiform activity,(Scheffer et al. 2017) potentially leading to inappropriate management.

Our study also identified clinical factors associated with diagnostic yield. Children with seizure onset before 12 months had a higher probability of a molecular diagnosis ($P = 0.028$), consistent with prior DEE studies.(Bashiri et al. 2023; Çapan Ö et al. 2024; Kocaaga and Yimenicioglu 2023; Sunnetci-Akkoyunlu et al. 2025; Vetri et al. 2024; Zou et al. 2024) Earlier identification may enable precision treatment, avoidance of contraindicated medications, and more accurate prognostic and recurrence-risk counseling. Although diagnostic yield declined with onset after 1 year, it remained substantial (approximately 42%), indicating that WES retains meaningful clinical utility beyond infancy.

Children classified as having intractable seizures with possible symptomatic epilepsy based on subtle neuroimaging abnormalities (eg, possible focal cortical dysplasia) or suspected but undocumented prior

brain insults had a lower diagnostic yield (P = 0.027). Prior studies suggest that when a structural etiology is clearly established, the contribution of monogenic causes may be reduced.(Tsang et al. 2019; Zhang et al. 2020) In practice, however, perinatal and early-life histories are often incomplete in referral centers, making causal attribution challenging.

Infantile spasms were also associated with a lower diagnostic yield in our cohort. This is biologically plausible given the broad etiologic spectrum of epileptic spasms, which includes structural causes (eg, malformations of cortical development), neurocutaneous disorders (eg, tuberous sclerosis complex), chromosomal abnormalities (eg, trisomy 21), and metabolic conditions.(Lux and Osborne 2004; Wirrell et al. 2015) Because many of these identifiable etiologies were excluded by our enrollment criteria, our findings suggest that, among children with uncontrolled infantile spasms and unremarkable magnetic resonance imaging, singleton WES may have a comparatively lower yield in this clinical context.

We also evaluated additional clinical predictors, including associated symptoms and signs, seizure type, and EEG features; however, none showed a statistically significant association with diagnostic yield in our cohort. Importantly, these analyses were conducted within a selected population of children with DRE, intellectual disability, and DEE. These predictors should not be generalized to unselected epilepsy populations. Predictors of diagnostic yield may differ in broader epilepsy cohorts, and future studies in general epilepsy populations are needed to better define yield-associated factors across the full clinical spectrum.

Despite excluding known inborn errors of metabolism at enrollment, we identified several neurometabolic disorders, including pathogenic variants in *ASS1*, *ATP7A*, and *GLDC*. Notably, *ATP7A*-related Menkes disease and *GLDC*-related nonketotic hyperglycinemia are not detected by routine newborn screening, whereas *ASS1*-related citrullinemia may be missed in individuals born before newborn screening was implemented. These findings underscore that neurometabolic disorders should remain in the differential diagnosis for children presenting with drug-resistant seizures and support the use of targeted metabolic testing when initial screening is unremarkable.

Clinical utility was substantial. Among genetically diagnosed patients, 34.1% underwent modifications to treatments currently available in Thailand, 15.9% were eligible for newly approved therapies not yet available locally, 34.1% required enhanced surveillance for comorbidities, 90.9% received genotype-informed prognostic counseling, and 18.2% obtained actionable reproductive counseling for family planning. We also observed a substantial delay between seizure onset and molecular diagnosis, with a median time to diagnosis of 81 months (IQR, 33–131; Table 1), likely driven by financial barriers to early genetic testing. The comparatively lower rate of family-planning decisions may reflect the limited availability of molecular diagnostics in the past. Earlier integration of genomic evaluation may shorten the diagnostic odyssey and reduce inappropriate treatment, particularly in resource-limited settings. Notably, 15.9% of genetically diagnosed children were potential candidates for emerging genotype-directed therapies, underscoring the value of longitudinal data registries to support future access planning, outcome monitoring, and implementation of precision treatments as they become available.

Strengths

This study has several strengths, including one of the largest prospective cohorts of DEEs reported from Southeast Asia. The prospective design with standardized phenotypic ascertainment reduced misclassification that commonly affects retrospective genetic epilepsy cohorts. Multidisciplinary variant interpretation, integrating neurologists, clinical geneticists, and variant scientists with systematic parental segregation testing where feasible, enhanced the reliability of pathogenicity classifications, particularly for reclassified VUSs. Systematic evaluation of clinical actionability across treatment, surveillance, prognostic, and reproductive domains provides a comprehensive assessment of real-world genomic medicine utility in a resource-limited setting.

Limitations

Limitations include the single-center design at a tertiary referral institution, which may introduce ascertainment bias toward more severe or diagnostically challenging phenotypes and limit generalizability to the broader Thai pediatric epilepsy population. Parental segregation testing was incomplete in some cases due to family unavailability, which may reduce VUS resolution. Finally, VUS reclassifications in this study were based on clinical and segregation evidence without functional validation, and some reclassifications may require reassessment as gene-disease evidence matures.

CONCLUSION

Singleton WES in Thai children with DRE and suspected DEE yields a high diagnostic rate with substantial clinical impact. Diagnostic probability is higher in early-onset epilepsy, yet actionable findings extend across age groups. Ion channel genes remain predominant; although marked molecular heterogeneity persists. Careful interpretation of structural imaging and incorporation of parental testing enhance diagnostic precision. Early genomic integration into routine pediatric epilepsy care in Southeast Asia may shorten the diagnostic journey and support precision medicine.

Declarations

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AUTHOR CONTRIBUTIONS

MC conceived and designed the study and acquired funding. PS, WJ, PP, PK, CSu, CSa, TT and UI contributed to patient recruitment and clinical data collection, with CSu obtaining informed consent and providing pretest genetic counseling. PP and PK contributed to clinical interpretation. WJ, NR, TP and CL performed variant interpretation and validation; WJ additionally conducted parental testing and prepared the initial tables. MC, NV performed the statistical analysis, data curation and data visualization. SL and

CL supervised the project. PS wrote the first draft of the manuscript and MC contributed substantive revisions; all authors critically revised and approved the final version of the manuscript.

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DATA AVAILABILITY STATEMENT

The de-identified data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

All authors declare that they have no personal or professional conflicts of interest relating to this study.

Ethical approval

This study was performed in line with the principles of the Declaration of Helsinki and the International Conference on Harmonisation–Good Clinical Practice guidelines. Approval was granted by the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University (protocol number 513/2567 [IRB1]; approval number Si-625/2024).

Consent to participate

Informed consent was obtained from all individual study participants or their guardians.

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Figures

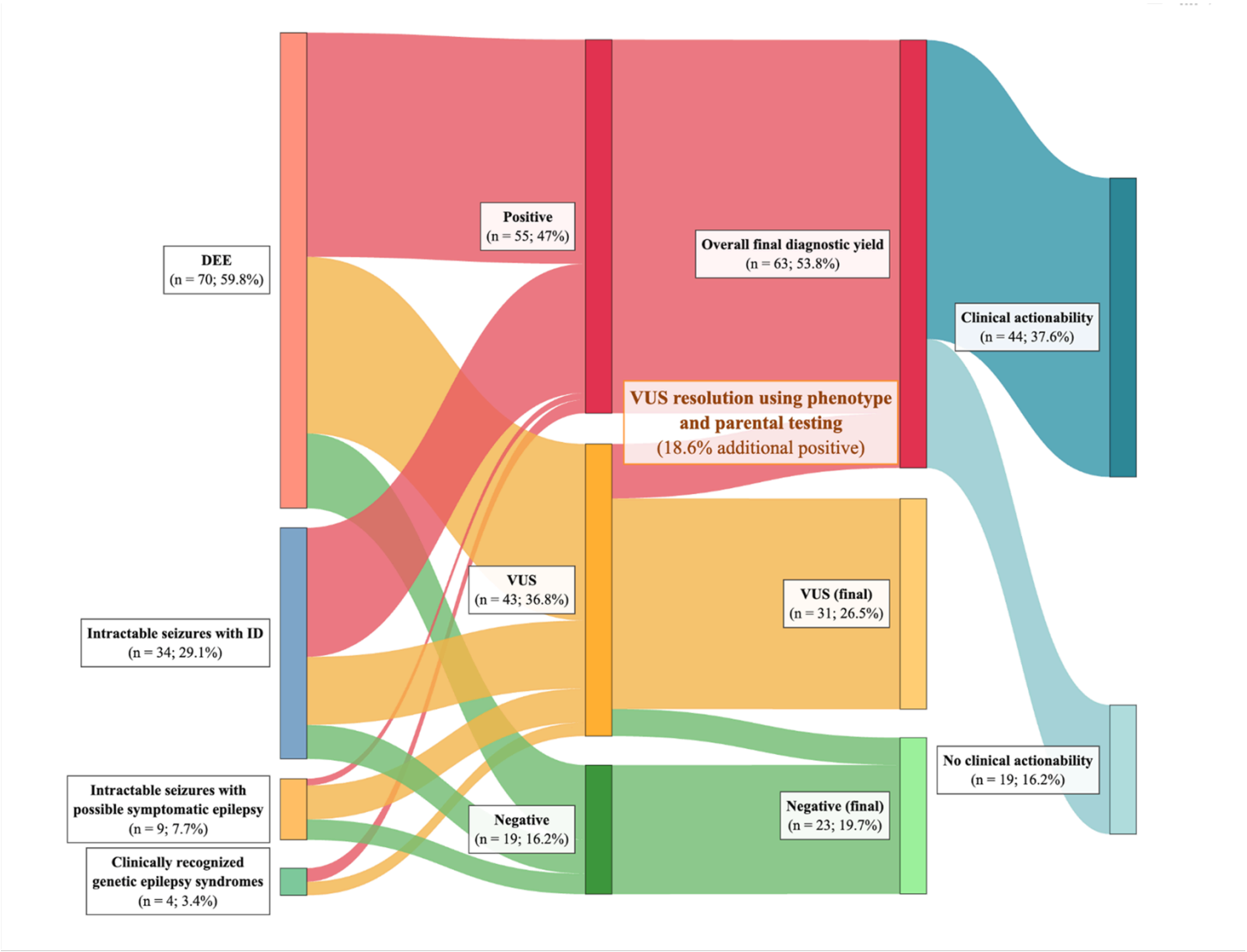


Figure 1

Sankey diagram illustrating the flow of patients from clinical phenotypic subgroups through whole-exome sequencing results to clinical-actionability outcomes.
Each row on the left represents a phenotypic subgroup with the number of patients (*n*) and the proportion classified as positive (pathogenic or likely pathogenic) on initial whole-exome sequencing. The middle column shows initial classification as positive, variant of uncertain significance (VUS), or negative; the highlighted box indicates the proportion of VUSs reclassified as positive after detailed phenotype review and parental segregation testing. The right column shows the final overall diagnostic yield and the proportion of patients in whom the result prompted a change in clinical management.
Abbreviations: DEE, developmental and epileptic encephalopathy; DRE, drug-resistant epilepsy; ID, intellectual disability; VUS, variant of uncertain significance.

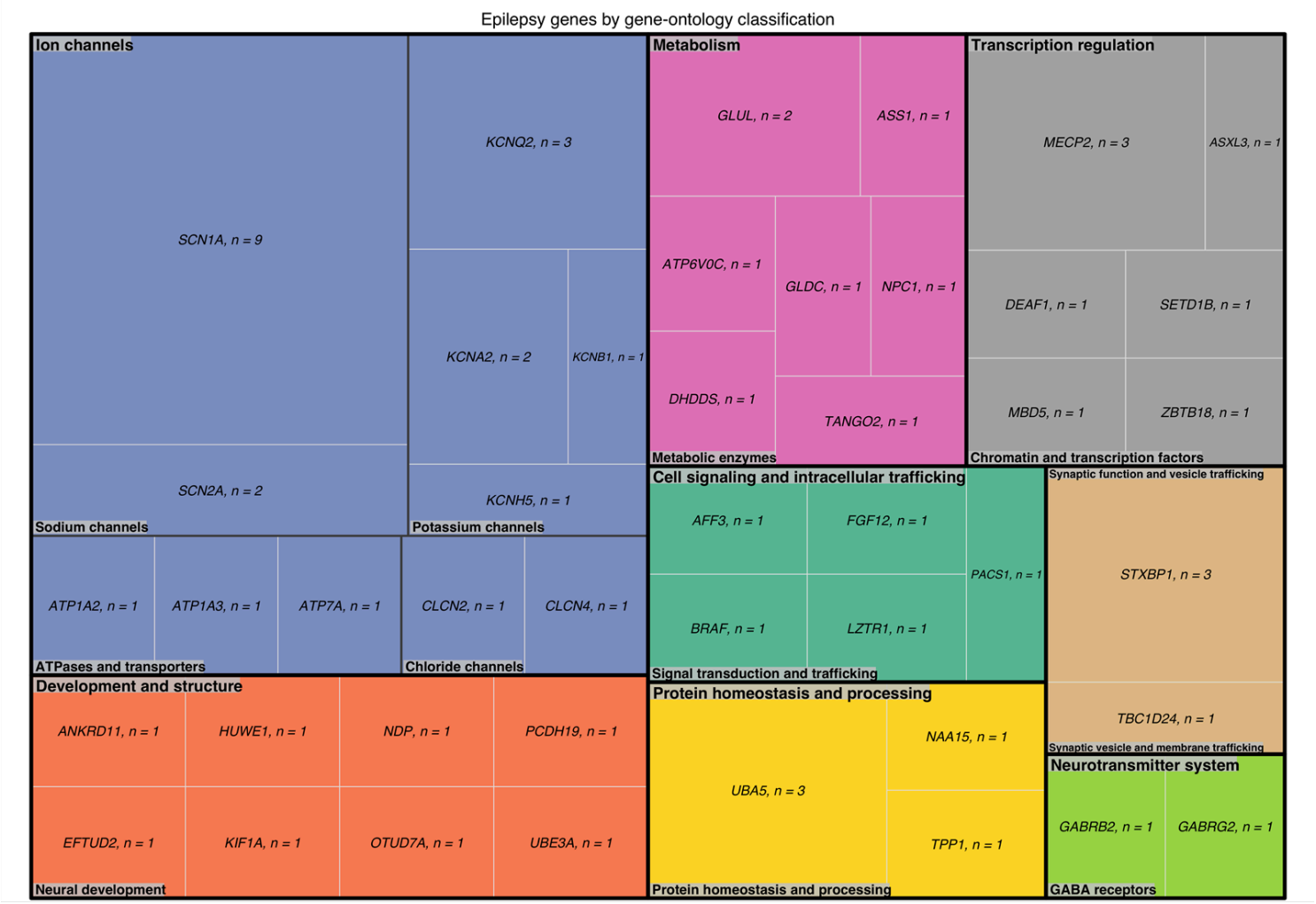


Figure 2

Treemap representation of genes identified by whole-exome sequencing in the diagnosed cohort. Each tile represents an implicated gene; tile area is proportional to the number of patients carrying a pathogenic or likely pathogenic variant in that gene (count shown in parentheses after the gene symbol). Genes are grouped by gene-ontology category (ion channels, metabolism, transcription regulation, cell signaling, synaptic function, development and structure, protein homeostasis, and neurotransmitter system) and, where applicable, by sub-category.

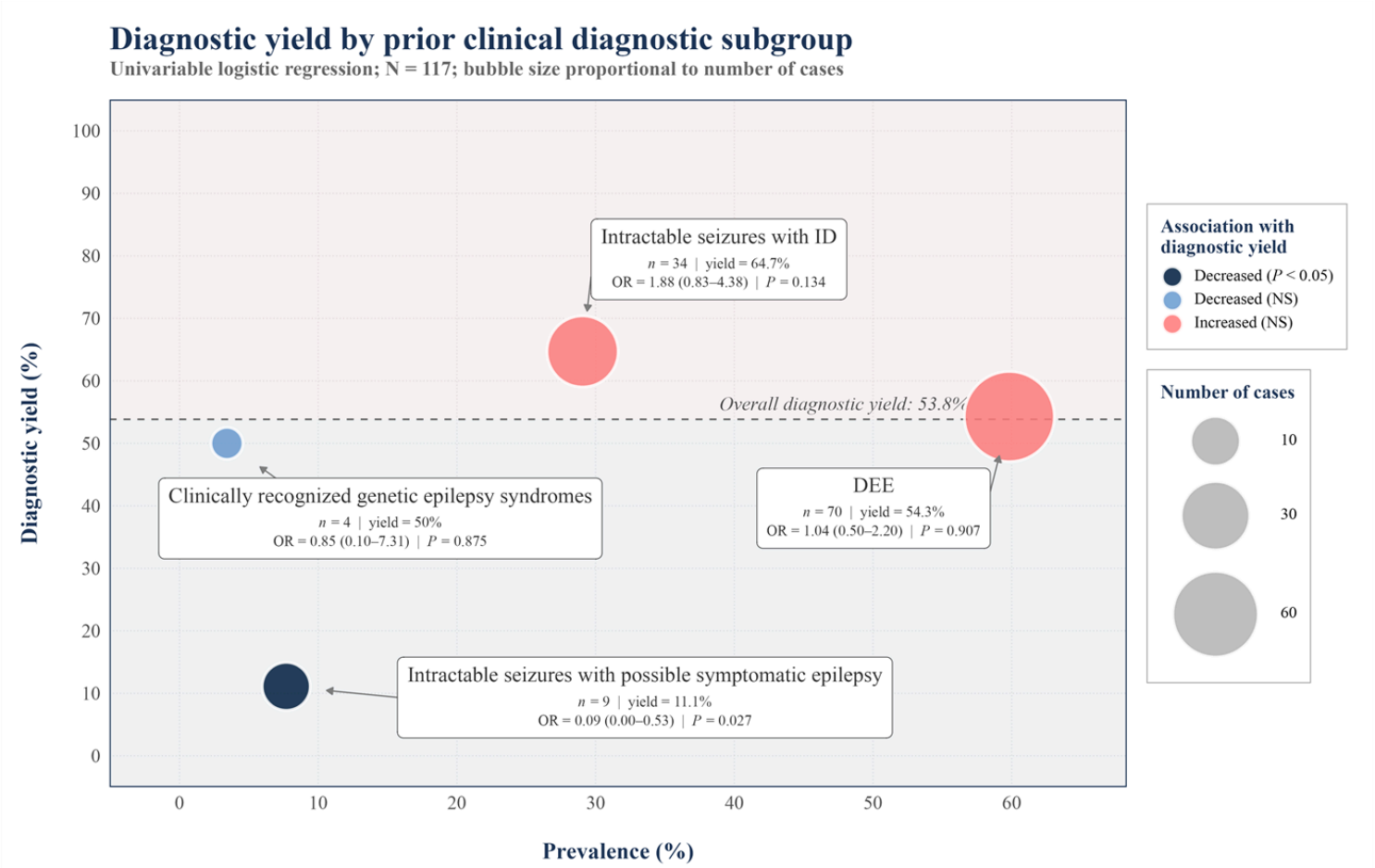


Figure 3

Bubble plot showing the association between the prior clinical diagnostic subgroup and diagnostic yield in 117 children with developmental and epileptic encephalopathy and drug-resistant epilepsy.
Bubble size is proportional to the number of patients in each subgroup, and the dashed horizontal line represents the overall diagnostic yield of 53.8%. Bubble color indicates the direction of the univariable logistic-regression association with the overall yield: pink for an increased point estimate (not statistically significant), light blue for a decreased point estimate (not statistically significant), and dark blue for a decreased point estimate that is statistically significant ($P < 0.05$). For each subgroup, the OR (95% CI) and *P* value from univariable logistic regression are shown in the corresponding callout.
Abbreviations: CI, confidence interval; DEE, developmental and epileptic encephalopathy; ID, intellectual disability; NS, not significant; OR, odds ratio.

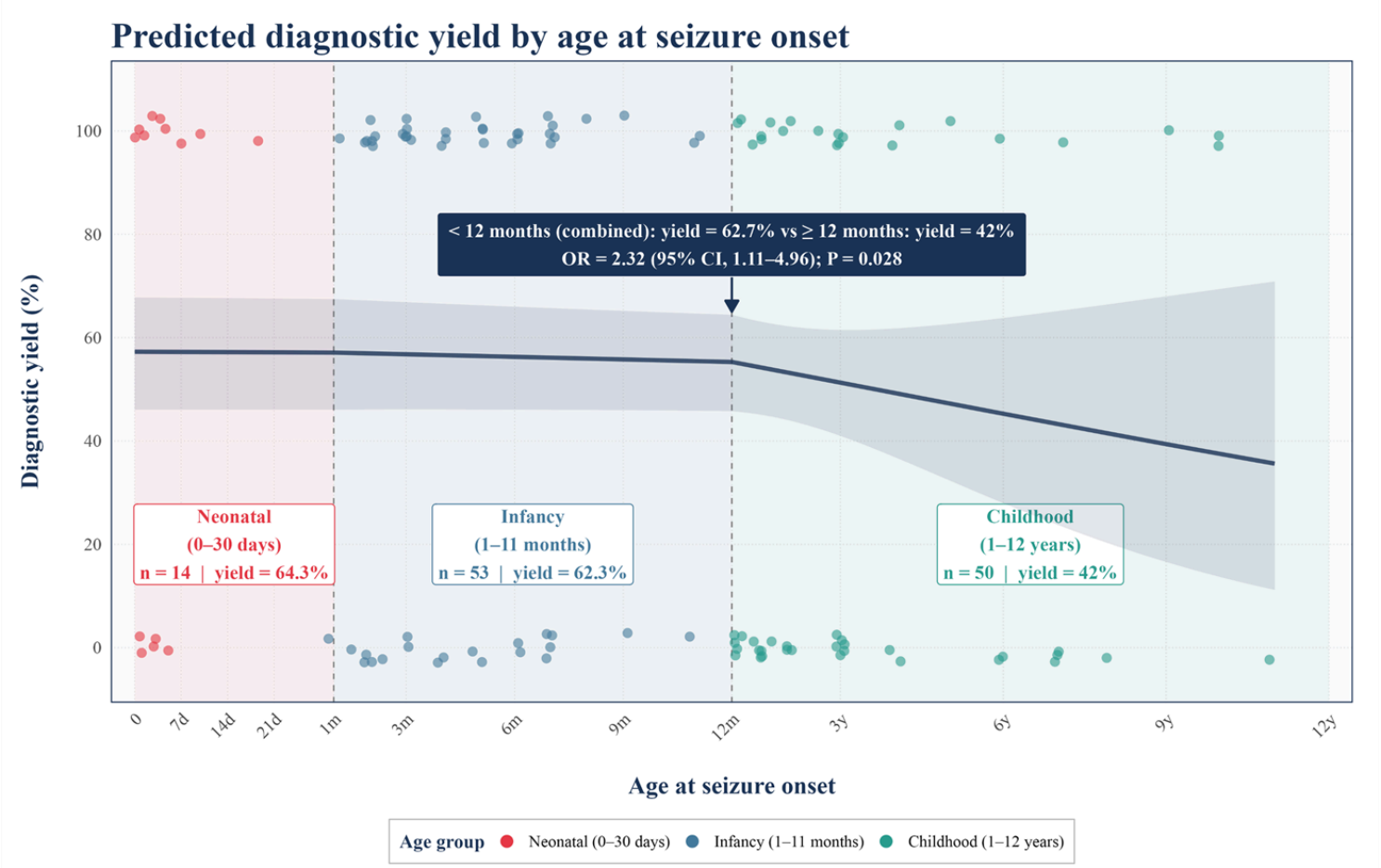


Figure 4

Predicted diagnostic yield as a function of age at seizure onset, with overlaid age-group strata.
The solid line indicates the predicted probability of a positive whole-exome sequencing result from a univariable logistic regression model, with the shaded area representing the 95% confidence interval. Dots represent individual patients (jittered vertically by outcome status); dashed vertical lines indicate age-group boundaries separating neonatal (0–30 days), infancy (1–12 months), and childhood (1–12 years) onset.
Within-strata yields are shown in the inset boxes (neonatal: 64.3%; infancy: 61.1%; childhood: 42%). The dichotomous comparison of < 12 months versus ≥ 12 months yielded an OR of 2.32 (95% CI, 1.11-4.96; *P* = 0.028).
Abbreviations: CI, confidence interval; OR, odds ratio.

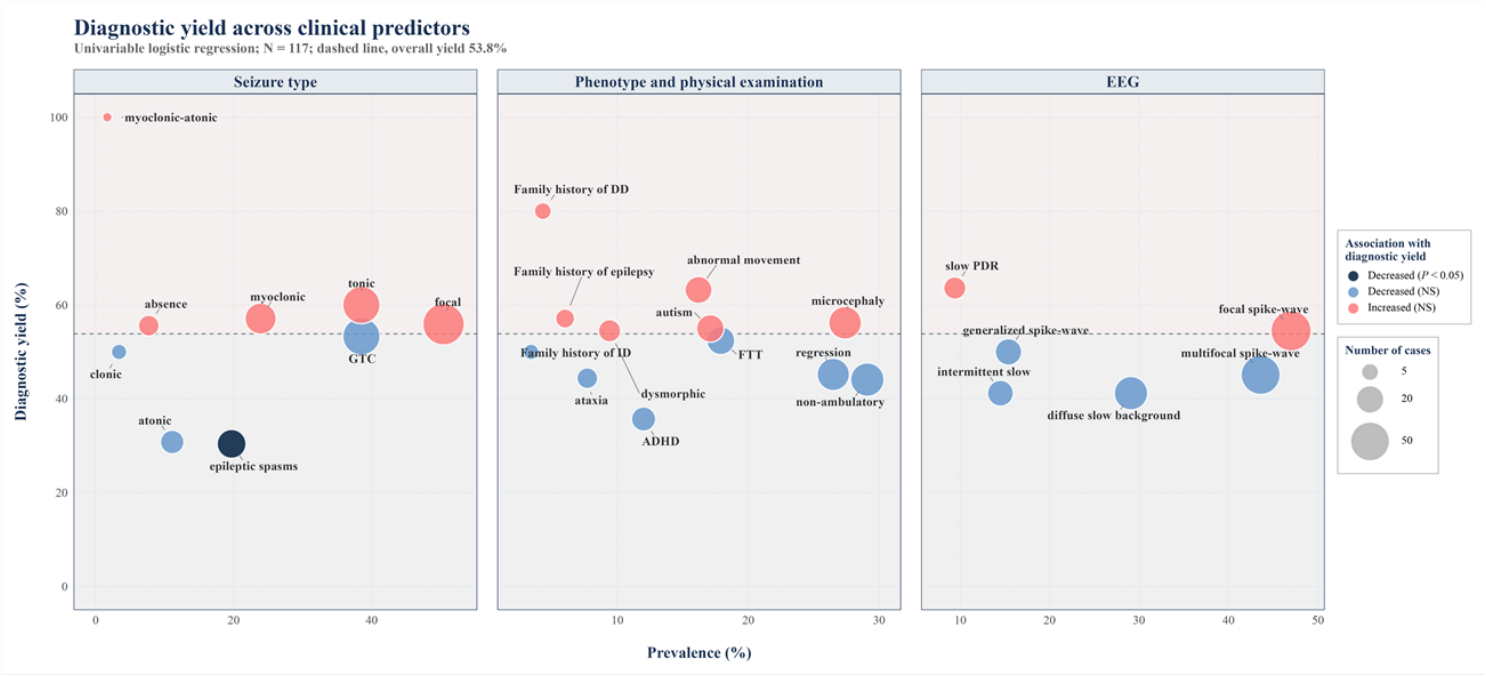


Figure 5

Diagnostic yield across clinical predictors in children with DEE and DRE.
Bubble plots illustrate subgroup prevalence (x-axis) and diagnostic yield (y-axis) for seizure types, phenotypic and physical examination features, and EEG findings. Bubble size reflects subgroup size. The dashed horizontal line represents the overall diagnostic yield of the cohort (53.8%). Bubble color indicates the direction of association with diagnostic yield in univariable logistic regression: pink, increased point estimate (not significant); light blue, decreased point estimate (not significant); dark blue, decreased point estimate with $P < 0.05$.
Abbreviations: ADHD, attention-deficit/hyperactivity disorder; CI, confidence interval; DD, developmental delay; DEE, developmental and epileptic encephalopathy; DRE, drug-resistant epilepsy; EEG, electroencephalography; FTT, failure to thrive; GTC, generalized tonic-clonic; NS, not significant; OR, odds ratio; PDR, posterior dominant rhythm.

Supplementary Files

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- HumanGeneticSupTab1.docx