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Therapeutic implications of etiology-specific diagnosis of early-onset developmental and epileptic encephalopathies (EO-DEEs): A nationwide Turkish cohort study

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Highligts

- Gene-related EO-DEEs are 48.3 % in the nationwide-Turkish cohort.
- Common non-structural genetic variants are *SCN1A, CDKL5, STXBP, KCNQ2*, and *PCDH19*.
- Ultra-rare variants of gene-related EO-DEEs are 52 % in the next-generation sequencing era.

- Therapeutic yield of genetic testing was 33.1 % in the NGS era in EO-DEEs.
- A step-wise diagnostic model indicated early implementation of WES in EO-DEEs.

Abstract

Objective

To evaluate the etiology-specific diagnosis of early-onset developmental epileptic encephalopathies (EO-DEEs) in a nationwide Turkish cohort to determine the implications for therapeutic management.

Methods

The cohort comprised 1450 patients who underwent EO-DEE. The utility of genetic testing was assessed with respect to the initial phases of next generation sequencing (NGS) (2005–2013) and the current NGS era (2014–2022). A predefined four-stepwise diagnostic model was evaluated using cost-effectiveness analysis. The diagnostic and potential therapeutic yields of the genetic tests were subsequently determined.

Results

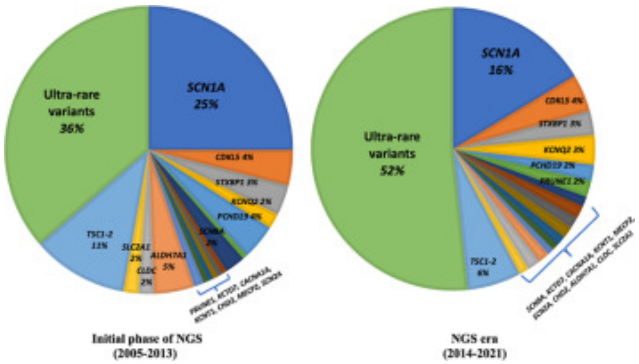
Gene-related EO-DEEs were identified in 48.3 % (n = 701) of the cohort: non-structural genetic (62.6 %), metabolic genetic (15.1 %), and structural genetic (14.1 %). The most common nonstructural genetic variants were *SCN1A* (n = 132, 18.8 %), *CDKL5* (n = 30, 4.2 %), *STXBP1* (n = 21, 2.9 %), *KCNQ2* (n = 21, 2.9 %), and *PCDH19* (n = 17, 2.4 %). The rate of ultra-rare variants (< 0.5 %) was higher in the NGS era (52 %) than that in the initial phase (36 %). The potential therapeutic yields with precision therapy and antiseizure drug modification were defined in 34.5 % and 56.2 % in genetic-EO-DEEs, respectively. The diagnostic model provided an etiology-specific diagnosis at a rate of 78.7 %: structural (nongenetic) (31.4 %), genetic (38.5 %), metabolic (6.1 %), and immune-infectious (2.8 %). Based on a cost-effectiveness analysis, the presented diagnostic model indicated the early implementation of whole-exome sequencing for EO-DEEs.

Significance

In the present cohort, the higher rate (48.3 %) of gene-related EO-DEE diagnoses in the NGS era provides a potential therapeutic management plan for more patients.

Graphical abstract

Graphic 1: The landscape of gene-related EO-DEEs in nationwide-Turkish cohort; initial phase of NGS (2005–2013) versus NGS Era (2014–2021)



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Keywords

Early-onset developmental epileptic encephalopathies; Genetic testing; Stepwise diagnostic model; Precision medicine

1. Introduction

The term developmental epileptic encephalopathy (DEEs), referring to the neurobiological process underlying epilepsy and the impact of both seizures and interictal epileptiform activity on cognitive function [[1], [2], [3]], was recently proposed by the International League against Epilepsy (ILAE). The term early onset DEE (EO-DEE) describes severe interictal epileptiform discharge and repeated seizures that commence within the first three years of life [4].

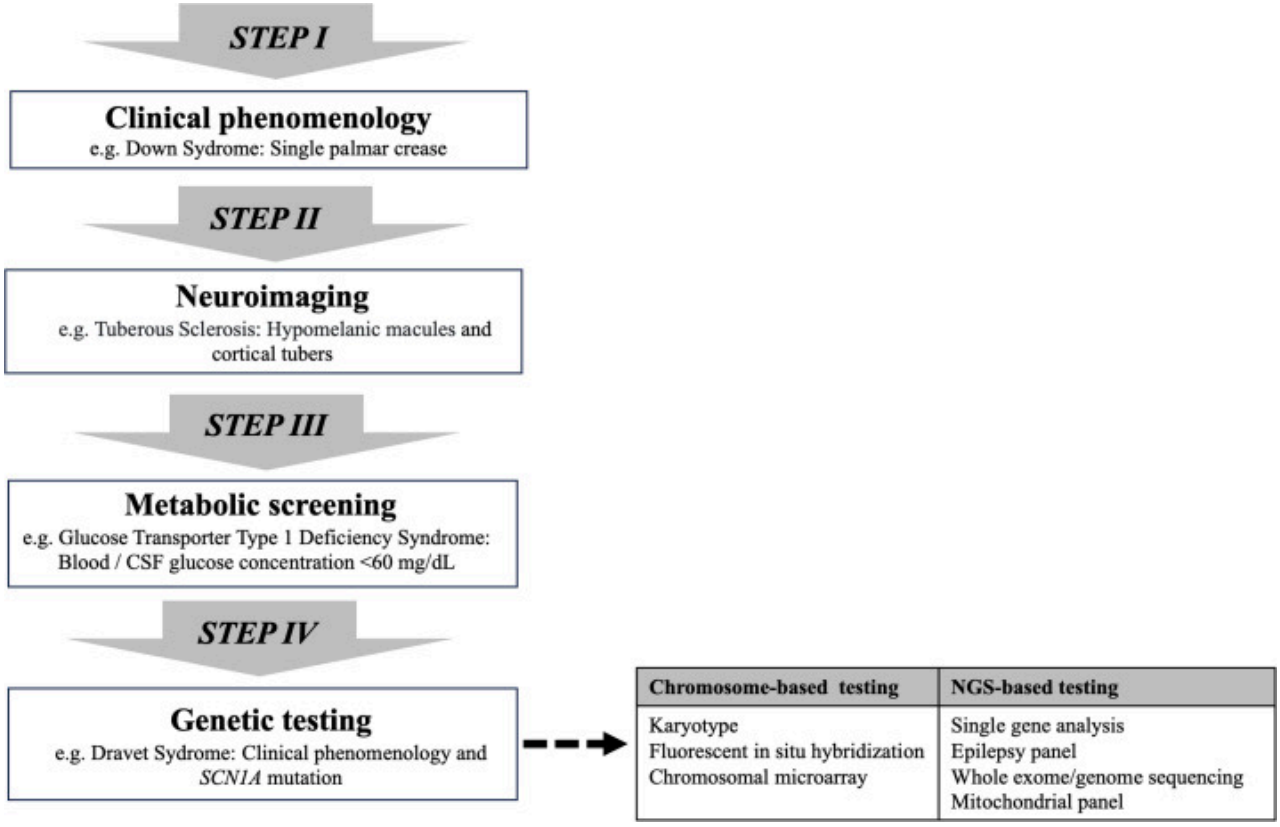
Early identification of the etiology-specific diagnosis of DEEs is crucial, not only for managing epileptic seizures, but also to prevent disease progression by modifying its trajectory. In the presence of an etiology-specific diagnosis, targeted therapy can be provided to patients with EO-DEE, such as antiseizure drug modification, early application of tailored epilepsy surgery, and the implementation of precision therapy. With the widespread adoption of advanced genetic testing methods such as next-generation sequencing (NGS), including epilepsy panels, and whole exome and genome sequencing (WES/WGS), the prevalence of etiology-specific diagnoses of DEEs has increased in recent years. [5,6]. Specifically, over the past ten years, there has been a notable change in the manner in which we understand DEEs. Many individuals who previously had an unknown etiology of their condition have now been diagnosed with genetic disorders. Recent research has revealed a growing range of genetic variations associated with DEEs [[7], [8], [9]].

The objectives of this nationwide cohort study were as follows: (1) to define gene-related EO-DEEs in the genomic era for potential therapeutic management, and (2) to investigate the diagnostic yields of a predefined four-stepwise diagnostic model for etiology-specific diagnosis with cost-effectiveness evaluation.

2. Patients and methods

2.1. Study cohort

This nationwide Turkish cohort study included 1450 patients with EO-DEE from 15 third-level medical centers in Turkey. This study was reviewed and approved by the Ege University Health Sciences Research Ethics Board . A restructured patient evaluation form, including a predefined four-stepwise diagnostic model for etiology-specific diagnosis, was provided to the centers. The stepwise diagnostic model was explained to the researchers at a Zoom meeting (Fig. 1). The inclusion criteria were: patients with a diagnosis of EO-DEEs (aged 0–36 months) with neurodevelopmental delay associated with EEG abnormalities The exclusion criterion was a diagnosis of well-defined self-limited infantile epilepsy without DEE.



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Fig. 1. The flow chart for the predefined four-stepwise diagnostic model for etiology-specific diagnosis in EO-DEEs.

2.2. The utility of a predefined four-stepwise diagnostic model for etiology-specific diagnosis

The stepwise diagnostic model comprised four distinct stages (Fig. 1). The principal investigators (SK and HT) provided online instructions on the stepwise diagnostic model and case report forms to all participating centers prior to commencing the study. The diagnostic yields of each step were defined using a form filled in by the local team that already contained all the information, while the principal investigators evaluated the steps necessary to make the diagnosis.

Clinical phenomenology (Step I). Patient files were reviewed to ascertain the patients' prenatal, perinatal, and postnatal histories. Information regarding genetic stigma and skin abnormalities related to neurocutaneous disorders was extracted from these files. In the first step, an etiology-specific diagnosis was defined solely based on clinical phenomenology.

Neuroimaging (Step II). Pediatric neurologists made the final interpretation of etiology-specific features based on magnetic resonance imaging (MRI) examinations, including central nervous system infections, hypoxic-ischemic brain injury, malformations of cortical development, tubers, and any clinicopathological findings (single brain cyst, delayed myelination, and mild cortical atrophy).

Metabolic screening (Step III). Metabolic screening was categorized into two groups: basic metabolic tests, which included ammonia, lactic acid, pyruvic acid, biotinidase, blood and urine amino acids, urine organic acids, carnitine profile, enzyme level, and activity; and comprehensive metabolic tests, which included a very long-chain fatty acid, lysosomal panel, cerebrospinal fluid amino acids, and cerebrospinal fluid neurotransmitters.

Genetic testing (Step IV). Genetic testing was conducted using both chromosome-based (karyotyping, fluorescent in situ hybridization, and chromosomal microarray) and NGS-based (single gene analysis, epilepsy panels, and WES) methods, depending on the clinical requirements. Genetic etiology-specific diagnosis refers to a comprehensive genetic testing process that includes initial and/or further genetic tests, along with genetic counseling. A predefined stepwise diagnostic model was applied by expert pediatric neurologists from all centers to code the etiology-specific diagnosis for each patient. Using a standardized stepwise diagnostic model, two independent pediatric neurologists (SK and YA) managed the etiology-specific diagnoses of each patient. The final judgment was made by a senior author (HT), when the two researchers outlined distinct steps. Four of the 15 institutions were able to implement the intended stepwise approach for patients with DEEs of all etiologies (n = 1322). The remaining 11 centers provided data exclusively for patients with DEE, identified using a stepwise diagnostic model.

2.3. Genetic testing and yields

NGS was initially introduced nationwide in Turkey in 2005. The initial phase of NGS platforms, spanning from 2005 to 2014, is mostly known for their reduced efficiency, high prices, and elevated error rates. Following the conclusion of the early NGS era, the use of more sophisticated algorithms, robust computing infrastructure, and skilled bioinformatics techniques has become prevalent, signifying a shift from the "initial phase of the NGS period" to a period characterized by the widespread adoption of more powerful and efficient NGS technologies. The time intervals in our investigation were determined according to the advances in NGS technology. In both the initial phase of NGS and NGS-era periods, chromosome-based tests, such as karyotyping, fluorescent in situ hybridization (FISH), and chromosomal microarray as well as NGS-based tests, such as single gene analysis, epilepsy panels, and WES, were used to different extents, depending on their availability. Due to the multicenter design of the study, the same kits and/or laboratory facilities could not be used for genetic testing at the centers. The results of the genetic tests varied temporally. The time periods of the study were planned according to the changes in genetic tests. For example, the content of the epilepsy panels in our center changed according to the study period, as follows: Early infantile epileptic encephalopathy panels containing 12 genes between 2010 and 2013: (*ARX, CDKL5, GABRG2, KCNQ2, PCDH19, SCN1A, SCN1B, SCN2A, SCN9A, SCN1A, SLC25A22, STXBP1*) and large epilepsy panels

containing 53 genes between 2014 and 2021 (*AARS, ALDH7A1, ALG13, ARHGEF9, ARX, ARV1, CDKL5, CACNA1A, DBH, DCX, DNM1, DOCK7, EEF1A2, FOLR1, FRRS1 L, GABRA1, GABRB3, GCH1, GNAO1, GRIN2B, GUF1, HCN1, ITPA, KCNA2, KCNB1, KCNQ2, KCNQ3, KCNT1, MAOA, NECAP1, PCDH19, PIGA, PLCB1, PNKP, PNPO, SCN1A, SCN2A, SCN8A, SCN9A, SLC2A1, SLC1A2, SLC12A5, SLC13A5, SLC25A12, SLC25A22, SLC35A2, SPTAN1, STXBP1, ST3GAL3, SZT2, TBC1D24, TH, WWOX)*.

WES conducted from 2014 to 2021 exclusively encompassed the nuclear genes assessed in Turkey. Since 2021, trio-based WES, which involves copy number variation (CNV) analysis, has gained popularity. Consequently, this study included patients who underwent monitoring until 2021.

The cohort was classified into four chronological periods, based on the development of genetic analysis: (I) 2005–2009, (II) 2010–2013, (III) 2014–2017, and (IV) 2018–2021. An additional dichotomy was implemented: the initial phase of NGS (from 2005 to 2013, *I* + II) and the NGS era (from 2014 to 2021, III + IV).

Despite alterations throughout different temporal phases, genetic consultations were conducted for all patients. Genetic testing in selected cases was carried out along with genetic counseling, involving professionals such as pediatric neurologists and geneticists. Pediatric neurologists at all institutes applied chromosomal assays, including karyotyping, FISH, and chromosomal microarray, for the initial evaluation of epileptic patients with or without developmental delay who were chosen for genetic testing. When a unique epilepsy phenotype was identified based on clinical features and EEG patterns, the initial investigation typically involves either single-gene analysis or epilepsy panels.

The diagnostic yields of genetic tests in the nationwide Turkish EO-DEE cohort were evaluated using data from centers that applied only the predefined four-stepwise diagnostic model, and were determined by identifying pathogenic variations that precisely corresponded to the phenotypic description of the patients.

Segregation analysis involves the application of genetic models to data on the phenotypes of family members. The objective was to evaluate hypotheses regarding the potential of one or more main genes and/or polygenes to explain the observed pattern of familial aggregation and mode of inheritance and to estimate the parameters of the best-fitting genetic model without the use of molecular data. The American College of Medical Genetic guidelines were applied to classify the variants as follows: class 5 (pathogenic), class 4 (presumably pathogenic), class 3 (unknown clinical significance-VUS), class 2 (probably benign), or class 1 (benign) [10]. Parental segregation analysis was conducted to ascertain whether the variant was inherited or whether it arose de novo. The identification of a compatible pathogenic or probable pathogenic variant was used to determine the genetic etiology. In specific instances, genetic diagnosis was supplemented with trio-based WES, WGS, and multiplex ligation-dependent amplification. The diagnostic yields of the genetic tests were determined based on the pathogenic variants that were identified and completely matched the patients' phenotypic descriptions.

Furthermore, the potential therapeutic yield of genetic testing was assessed in relation to precision and empirical therapy (which involves prescribing a specific drug or avoiding substances that may worsen epileptic encephalopathy) in the genetic etiology-specific diagnosis group. Precision medicine is predicated on the concept understanding the function of mutated genes and implementing interventions that are either known or anticipated to rectify the dysfunction caused by

mutated gene products [11]. This information may be useful under various circumstances: First, the selection of antiseizure medications (ASMs) targeting the pathogenic mechanisms of the disease involves the logical utilization of current ASMs to address the molecular dysfunction caused by the mutant gene. For example, the *SCN8A*-related DEE phenotype results from a gain-of-function mutation in neuronal sodium channels. Sodium channel-blocking ASMs such as phenytoin and carbamazepine can effectively reduce seizures in these patients. These medications reduce the dysfunction caused by the affected channels. Secondly, the identification of molecular defects in genetic and functional studies has led to the development of targeted therapies based on novel molecules. The pathogenic mechanisms underlying *PCDH-19*-named DEE may entail a shortage of allopregnanolones in the central nervous system. This prompted a pilot study to investigate the potential therapeutic effects of ganaxolone, an analog of allopregnanolone, in patients with this disease [12].

Genetic test results were dichotomized into EO-DEEs using NGS and EO-DEEs using chromosome-based tests. The EO-DEEs with NGS were categorized into three functional etiology-specific subgroups; (1) 'non-structural genetic' refers to genetic disorders that originating from aberrations in ion transport, synaptic dysfunction, transporter defects, or transcriptional dysregulation (e.g. *SCN1A* variants), (2) 'metabolic-genetic' refers to metabolic pathway disorders affecting the metabolism and leads to brain dysfunction, which in turn causes seizures *(*e.g., *ALDH7A1* variants*),* (3) 'structural-genetic' refers to disorders triggered by genetic conditions that cause changes in the structure of the brain, leading to the development of epilepsy (e.g., tuberous sclerosis, and *DEPDC5* variants). Rare variants were defined as those accounting for 0.5–1 % of the presented cohort and are depicted in the pie graph. Variants with a rate < 0.5 % were defined as ultrarare variants.

2.4. Analysis of cost-effectiveness

The current Euro values for these tests were obtained from hospital bills utilizing electronic medical records, and the fees currently in effect were added. The aggregate cost of conducting cost analysis, magnetic resonance imaging, basal and advanced metabolic screening tests, and genetic testing was computed from these values. In chromosome-based genetic testing, a stepwise genetic test was performed when both karyotypes and chromosomal microarrays were conducted. Conversely, a non-stepwise genetic test was considered when a chromosomal microarray was performed without prior karyotyping. In -based tests were classified as stepwise if the analysis was performed on a single gene, followed by an epilepsy panel and/or WES. Tests were classified as non-stepwise if WES was performed at the initial stage. A cost comparison between the two approaches was conducted.

2.5. Statistical analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences 26.0. Descriptive statistics included the mean, standard deviation, and median (minimum-maximum) for continuous variables, and the number of patients and percentage for categorical variables. Furthermore, Pearson's chi-square test or Fisher's Exact Chi-Square test and Multivariate Analysis of Variance were applied to analyze categorical variables across different groups. The data are presented as frequencies and percentages. The results were deemed statistically significant at $p < 0.05$.

3. Results

3.1. Demographics

The study included 1450 children with EO-DEEs (male, 53.8 %; women, 46.2 %). During the study, the mean age at seizure onset was 7.2 ± 6.9 (0–30) months, while the mean follow-up duration was 4.7 ± 3.7 years. The time required to make an etiology-specific diagnosis was 11 months (range, 1–24 months).

3.2. EO-DEEs with genetic etiology

The rate of genetic etiology in the cohort was 48.3 %, as shown in Table 1 In our cohort of 701 patients who received a molecular diagnosis, pathogenic/likely pathogenic variants were identified in 203 genes associated with the developmental epileptic encephalopathy phenotype. The most common non-structural genetic variants were found in *SCN1A* (*n* = 132, 18.8 %), *CDKL5* (*n* = 30, 4.2 %), *STXBP1* (*n* = 21, 2.9 %), *KCNQ2* (*n* = 21, 2.9 %), *PCDH19* (*n* = 17, 2.4 %), *ALDH7A1* (*n* = 15, 2.1 %), *PRUNE1* (*n* = 11, 1.5 %), *SCN8A* (*n* = 10, 1.4 %), *KCTD7* (*n* = 9, 1.2 %), *CACN1A* (*n* = 8, 1.1 %), and *KCNT1* (*n* = 8, 1.1 %) (Table 2, Graphic 1). The spectrum of rare variants (detected at a rate of 0.5–1 %) in the initial phase of NGS (*n* = 7, *PRUNE1, KCTD7, CACNA1A, KCNT1, MECP2, SCN2A, CHD2*) changed during the NGS era (*n* = 10, *SCN8A, KCTD7, CACNA1A, KCNT1, MECP2, SCN2A, CHD2, ALDH7A1, CLDC, SLC2A1*). The rate of ultra-rare variants (detected at a rate of < 0.5 %) was higher in the NGS era (52 %) than that in the initial phase (36 %) (Fig. 1).

Table 1. The genetic landscape of a nationwide-Turkish cohort with early-onset developmental epileptic encephalopathies (EO-DEE, *n* = 1450) for therapeutic implications.

Genetic-EO-DEEs *n* = 701 / 1450 (48.3 %)	**EO-DEEs with NGS *n* = 644**			**EO-DEEs with chromosome-based tests *n* = 57 (8.1 %)**
	Non-structural *n* = 439 (62.6 %)	**Structural disorders *n* = 99 (14.1 %)**	**Metabolic pathway *n* = 106 (15.1 %)**	
Initial phase of NGS (2005–2013) *n* = 176 (25.1 %)	*n* = 104 *SCN1A*#(44), PCHD19*(7), CDKL5*(7), STXBP1*# (6), SCN2A*# (2), SCN8A*(4), KCNQ2*(3), CHD2(3), CYFIP2 (2), ADAM22 (2), PLCB1(2), ARHGEF9*, ARID1B, CACNA1A*#, CAPN3, DIAPH1, FGF12*# GABRB3*, GNO1, GRIN1, KCNA1*#, KCNT1*#, KCNT2*#, KIF1A, MECP2*, MYH7, NECAP1, PNKP, PRUNE1, SCN2B*#, TBC1D24, UBA5, UNC80	*n* = 29 *TSC 1–2*#(20), DEPDC5*, DENND5A, IFH1, CLP1, RANBP2, SLC35A2, TSEN54, WDR45, WDR62	*n* = 26 *ALDH7A1*(8), SLC2A1*(3), GLDC*#(3), PEX1(2), ABCC8*, BTD*, CLN8, DPYS, EARS1*#, FOLR1*, HEXA, KCTD7, MT-ATP6, PNPO*	*n* = 17 Angelman synd. (7), Trisomy 21 (3), Prader Willi synd.(2), *Xq28 dup (2), 1p36 del, 21q22.12 dup,* Kabuki syndrome
NGS era (2014–2021) *n* = 525 (74.9 %)	*n* = 335 *SCN1A*#(88), CDKL5* (23), KCNQ2*(18), STXBP1*# (15), PCDH19*(10), PRUNE1(10), CACNA1A*#(7), KCNT1*#(7), SCN8A*(6), MECP2*(6), SPTAN(5), AP3B2(4), CHD2(4), DNM1(4), GRIN2A*(4), SCN2A*#(4), WWOX(4), CACNA1E*#(3), GNOA1(3), GRIN1*(3), SCN9A*#(3), UFM1(3), ADAM22(2),	*n* = 70 *TSC1–2*#(34), ARX*#(4), NF(4), CLP1 (3), OCLN(3), TREX1* (3), DEPDC*(2), PAFAH1B1(2),	*n* = 80 *GLDC*#(9), KCTD7(8), ALDH7A1*(7), SLC2A1*(6), GAMT*(4), MTHFR*(3),	*n* = 40 Trisomy 21 (16), Angelman syndrome (14), *14q del (2), 15q11 dup, 16q dup,*

Genetic-EO-DEEs *n* = 701 / 1450 (48.3 %)	EO-DEEs with NGS *n* = 644			EO-DEEs with chromosome-based tests *n* = 57 (8.1 %)
	Non-structural *n* = 439 (62.6 %)	Structural disorders *n* = 99 (14.1 %)	Metabolic pathway *n* = 106 (15.1 %)	
	ANK3(2), ATP1A3#(2), CACNA1H(2), CACNA2D2(2), EEF1A2(2), FOXG1(2), GABRB2#(2), GABRB3*(2), GRIA3(2), HNRNPU#(2), KCNA2*#(2), KCNQ1*(2), KMT2C(2), KMT2E(2), NAPB(2), RYR3#(2), SCN3A#(2), ST3GAL3(2), TBC1D24(2), AARS1, ACADM, ACTL6B, ADD3, ANKRD11, ANO10*, ARID1B, ASPM, ATP6V1A, BBS7, BRAF, CACNA1 G, CACNB4, CASCK, CNTNAP1, CPLANE1, EFEMP2, EIF2S3, ELP2, FRRS1L#,GABRA1#, GLRA1#, GRIN2B*, HCN1*, HFE, IQSEC2, ITPA, KANSL1, KCNQ3*, KIF1A, KCNJ10, KMT2A, MAP2K2, MBOAT, MED13 L, MED25, MFSD2A, NECAP1, NR2F1#, PACS2, PGAP2, PIGA*, PIGQ, PLA2G6, PLCB1, PNKP, POGZ, RARS2, RBL2, SCL25A22, SLC9A6, SCN2B#, SETD1B, SLC12A5, SLC13A5*#, SLC20A2, ST3GAL5, SZT2, TCF4, TRPM3, TTN.*	*CNTN2, CUX2, DYNC1H1, FARSB, IFIH1, LIS1, MAPK8IP3, NEDBASS, PIK3R2, TBCD, TNEM106B, TUBB2B, TUBGCP2, TUBGCP6, WDR62*	*ADSL (2), ALG11 (2), ASNS(2), COQ4* (2), FOLR1*(2), GM2A(2), HSD17B4(2), MMACHC*(2), PEX1(2), AMT*#, ASS1, ATP7A1*, BDT*, BRAT1#, DDC*, DPAGT1, EARS2#, GALC, GCH1*, HEXA, HMGCL, MCOLN1, MICOS13#, MOCS1#, MTO1#, NARS2#, NDUFA13#, NDUFS4#, PAH*, PDHA1, PDHX, SLC19A3*, UMPS, TH*	*16p11.2 del, 17p13.3 dup, 18q dup, 18p del 2q24.3-q31.3 del. 47, XXX*

(*): therapeutic yield with precision therapy was defined at 34.5 % (242/701) in genetic-EO-DEEs.

(#): therapeutic yield with empirical therapy with antiseizure modification was defined at 56.2 % (394/701) in genetic- EO-DEEs.

Table 2. Most commonly identified gene-named EO-DEEs in the whole cohort (*N* = 1450): the initial phase of NGS (from 2005 to 2013) versus the NGS era (from 2014 to 2021).

Genetic-EO-DEE *N* = 701	Genetic testing period, n		Genetic testing modalities for diagnosis (n,%)
SCN1A *N* = 132, 18.8 %	Initial phase of NGS	44	SGA (13, 29.5 %), EP (28, 63.6 %), WES (3, 6.8 %)
	NGS Era	88	SGA (28, 31.8 %), EP (43, 48.8 %), WES (16, 18.1 %), MLPA (1, 1.1 %)

Genetic-EO-DEE *N* = 701	Genetic testing period, n		Genetic testing modalities for diagnosis (n,%)
CDKL5 *N = 30, 4.2 %*	Initial phase of NGS	7	SGA (1, 14.2 %), EP (2, 28.5 %), WES (4, 57.1 %)
	NGS Era	23	EP (9, 39.1 %), WES (14, 60.8 %)
STXBP1 *N = 21, 2.9 %*	Initial phase of NGS	6	EP (4, 66.6 %), WES (2,33.3 %)
	NGS Era	15	EP (11, 73.3 %), WES (4, 26.2 %)
KCNQ2 *N = 21, 2.9 %*	Initial phase of NGS	3	EP (3, 100 %)
	NGS Era	18	EP (11, 61.1 %), WES (7, 38.8 %)
PCHD19 *N = 17, 2.4 %*	Initial phase of NGS	7	SGA (1, 14.2 %), EP (6, 85.8 %)
	NGS Era	10	SGA (1, 10 %), EP (8, 80 %), WES (1, 10 %)
PRUNE1 *N = 11, 1.5 %*	Initial phase of NGS	1	WES (1, 100 %)
	NGS Era	10	WES (10, 100 %)
SCN8A *N = 10, 1.4 %*	Initial phase of NGS	4	EP (2, 50 %), WES (2, 50 %)
	NGS Era	6	EP (4, 66.6 %) WES (2, 33.3 %)
KCTD7 *N = 9, 1.2 %*	Initial phase of NGS	1	WES (1, 100 %)
	NGS Era	8	EP (3, 37.5 %), WES (5, 62.5 %)
CACNA1A n = 8, 1.1 %	Initial phase of NGS	1	WES (1, 100 %)
	NGS Era	7	EP (2, 28.5 %), WES (5, 71.5 %)
KCNT1 *N = 8, 1.1 %*	Initial phase of NGS	1	WES (1, 100 %)
	NGS Era	7	EP (2, 28.5 %), WES (5, 71.5 %)
CHD2 *N = 7, 0.9 %*	Initial phase of NGS	3	WES (3, 100 %)
	NGS Era	4	WES (4, 100 %)
MECP2 *N = 7, 0.9 %*	Initial phase of NGS	1	SGA (1, 100 %)
	NGS Era	6	SGA (3, 50 %), EP (1,16.6 %), WES (2,33.3 %)
SCN2A *N = 6, 0.8 %*	Initial phase of NGS	2	EP (2, 100 %),
	NGS Era	4	EP (1, 25 %), WES (3, 75 %)
ALDH7A1 *N* = 15, 2.1 %	Initial phase of NGS	8	SGA (4, 50 %), EP (3, 37.5 %), WES (1, 12.5 %)
	NGS Era	7	SGA (2, 28.5 %), EP (3, 42.8 %), WES (2, 28.5 %)
CLDC *N* = 11, 1.5 %	Initial phase of NGS	3	SGA (3100 %)
	NGS Era	8	SGA (6, 75 %), WES (2,25 %)
SLC2A1 *N = 9, 1.2 %*	Initial phase of NGS	3	SGA (1, 33.3 %), MLPA (1, 33.3 %), WES (1, 33.3 %)
	NGS Era	6	SGA (4, 66.6 %), WES (2, 33.3 %)

Genetic-EO-DEE *N* = 701	Genetic testing period, n		Genetic testing modalities for diagnosis (n,%)
TSC1–2 *N* = 52, 7.4 %	Initial phase of NGS	19	SGA (16, 84.2 %)
	NGS Era	33	SGA (29, 87.8 %), EP (2, 6 %), WES (1,3 %)

NGS: Next-generation sequencing, EP: Epilepsy panel, WES: Whole exome sequencing, MLPA: Multiplex-ligation dependent probe amplification, SGA: Single gene analysis.

The most commonly identified genetic variants with respect to genetic testing modalities in both the initial phase of NGS and in the NGS era are presented in Table 2. The most prevalent genetically identified variants were similar in both periods (*SCN1A, CDKL5, STXBP1, KCNQ2*, and *PCDH19)* and were mainly identified in the epilepsy panels. The following genetic variants, *PRUNE1* (1.5 %), *SCN8A* (1.4 %), *KCTD7* (1.2 %), *CACNA1A* (1.1 %), *KCNT1* (1.7 %), *MECP2* (0.9 %), and *CHD2* (0.9 %), were mainly diagnosed based on WES analysis. The remaining variants, *SLC2A1, ALDH7A1, CLDC,* and *TSC1–2* were diagnosed using single-gene analysis under the guidance of clinical phenomenology (step I), neuroimaging (step II), and metabolic screening (step III), *TSC* (7.4 %), *CLDC* (1.5 %), *SLC2A1* (1.2 %), and *ALDH7A1* (2.1 %).

A test results potentially allowing precision therapy was achieved in 242 of the 701 (34.5 %) EO-DEEs with genetic etiology-specific diagnoses in the presented cohort through genetic testing. The potential therapeutic yield of precision therapy remained almost constant at 33.1 % during the NGS period (174/525), whereas it was 38.6 % (68/176) during the initial phase of NGS. Nevertheless, the therapeutic yield of empirical therapy was 56.2 % (394/701) in the genetic etiology-specific diagnosis group. The therapeutic yields of empirical therapy were comparable across the two follow-up periods, with values of 52.8 % (93/176) and 41.9 % (220/525), respectively.

3.3. Four-stepwise diagnostic model for EO-DEEs

An etiologic trend analysis was performed using a four-stepwise diagnostic model (Fig. 1) on 1322 patients from 11 centers. Table 3 illustrates the etiological trends in relation to the initial phase of NGS and the NGS era using the model.

Table 3. The etiology-specific diagnosis in a nationwide-Turkish EO-DEEs cohort in the four-stepwise diagnostic model.

n (%) Etiology-spesific diagnosis (ESD) n:1041/1322 (78.7)	Initial phase of NGS (2005–2013) 428 (32.4)	NGS era (2014–2021) 894 (67.6)	P
Structural (non-genetic) ♦415 (31.4)	151 (35.3)	264 (29.5)	<0.001*
Metabolic 80 (6.1)	20 (4.7)	60 (6.7)	
Immune/ Infectious 37 (2.8)	13 (3)	24 (2.7)	

n (%) Etiology-spesific diagnosis (ESD) n:1041/1322 (78.7)	Initial phase of NGS (2005–2013) 428 (32.4)	NGS era (2014–2021) 894 (67.6)	P
Genetic ♦509 (38.5)	128 (29.9)	381 (42.6)	
Unknown ♦ 281 (21.3)	116 (27.1)	165 (18.5)	

NGS: Next generation sequencing.

*
Chi-square test,.

♦
Manova test.

The stepwise diagnostic model defined an etiology-specific diagnosis in 78.7 % of the cohort EO-DEEs. Genetic etiology was the most prevalent etiologic category, with a rate of 38.5 % across the entire cohort. The extensive use of NGS-based assays resulted in a substantial increase in genetic etiology-specific diagnoses, from 29.9 % (initial phase of NGS) to 42.6 % (NGS era). Structural (non-genetic) and metabolic etiologies were identified in 31.4 % and 6.1 % of the entire cohort, respectively. However, the etiology was unknown in 281 (21.3 %) children. The extensive use of genetic testing significantly decreased the rate of unknown etiologies from 27.1 % (in the initial phase of NGS) to 18.5 % (in the NGS era) ($p < 0.001$). However, metabolic and immune/infectious etiologies persisted at comparable rates throughout the follow-up period (2005–2021). Additionally, the rates of genetic etiology increased considerably, whereas the rates of non-genetic structural etiology-specific diagnosis and unknown etiology decreased significantly between the follow-up periods ($p < 0.01$) (Table 3).

3.4. Utility of the four-stepwise diagnostic model for etiology-specific diagnosis

The cohort was divided into four categories based on the duration of the follow-up periods (Table 4): the initial phase of NGS (I. Period from 2005 to 2009 n = 178, 13.4 % and II. Period from 2010 to 2013 n = 250, 18.9 %); and the NGS era (III. Period from 2014 to 2017 n = 404, 30.5 % and IV. Period from 2018 to 2021 n = 492, 37.2 %).

Step I. Clinical phenomenology alone allowed an etiology-specific diagnosis in 24 patients (1.8 %). For specific clinical entities, including severe perinatal damage, TSC, and trisomies, a robust preliminary diagnosis could be made; however, the majority of patients with EO-DEE required genetic or radiological evidence to establish a definitive diagnosis.

Step II. The diagnostic yield of Step II for etiology-specific diagnosis was 27.4 %. Neuroimaging modalities (magnetic resonance imaging and computerized tomography) provided an etiology with a range of 26.4–28.5 % during the study period (periods I to IV). The rates of MRI abnormalities were 43.2 %, 35.2 %, 63.7 %, and

100 % in the genetic, unknown, metabolic, and immune/infectious etiology groups, respectively.

Step III. Metabolic screening (basic/comprehensive) resulted in an etiology-specific diagnosis in 15.7 % of the cohort (Table 4). Basic metabolic screening provided the etiology in 54.5 % of infants in the metabolic etiology cohort. Comprehensive metabolic testing was required for the remaining 45.4 % of patients. To investigate metabolic or immune/infectious causes, cerebrospinal fluid analyses were performed in 26.3 % (348/1322) of patients.

Step IV. With the extensive use of NGS modalities, the identification of genetic etiologies increased from 39.9 % to 66 %. The utility of genetic testing with respect to etiologic subgroups underwent a substantial transformation from the initial phase of NGS to the NGS era ($p < 0.01$) (Table 4). The structural (non-genetic) etiology and unknown groups underwent extensive genetic testing as well as reverse phenotyping with NGS modalities during the NGS era, in anticipation of a potential genetic etiology-specific diagnosis in the presence of negative results in the first three steps. The incidence of unknown etiologies decreased from 27.1 % to 18.5 %.

Table 4. The utility of the four-stepwise diagnostic model for the etiology-specific diagnosis of patients with EO-DEEs.

n:1322 (%)	**Initial phase of NGS n:428 (32.3)**		**NGS era n: 894(67.7)**		**p**
	I. Period (2005–2009) n:178 (13.4)	**II.Period (2010–2013) n:250 (18.9)**	**III.Period (2014–2017) n:404 (30.5)**	**IV.Period 2018–2021 n:492 (37.2)**	
Step I: clinical phenomenology only,n:24 (1.8)	4 (2.2)	6 (2.4)	4 (1)	10 (2)	*< 0.01**
Step II: neuroimaging n:362 (27.4)	47 (26.4)	67 (26.8)	115 (28.5)	133 (27.1)	
Step III: metabolic screening n:207 (15.7)	56 (31.5)	71 (28.4)	56 (13.9)♦	24 (4.9)+	
StepIV: genetic testing♦ n:729 (55.1)	71 (39.9)	106 (42.4)	228 (56.6)♦	324 (66)+	

NGS: Next generation sequencing, initial genetic testing in the first-line diagnostic work-up, *Chi-square test,♦+Manova test.

Genetic-EO-DEEs were diagnosed in 119 (41.7 %) of 285 patients using epilepsy panels, and in 251 (66.5 %) of 377 patients using WES. Genetic etiology was diagnosed in 100 (41.3 %) of the 242 patients examined using single-gene analysis. Patients with TSC or NF were excluded as they were diagnosed

based on clinical phenomenology and neuroimaging studies. However, chromosome-based tests and molecular genetic diagnosis were performed in 26 (6.7 %) of 384 patients by karyotyping, 25 (13.3 %) of 187 patients by chromosomal microarray, and 17 (18.6 %) of 91 patients by FISH.

The DEE cohort's aggregate diagnostic yield of genetic testing was 44.5 % (589/1322) when the stepwise diagnostic model was used. Between 2005 and 2021, the diagnostic yield of genetic testing increased from 31.3 % to 45.6 % over the four follow-up periods. Karyotyping, FISH, and chromosomal microarray assays, all of which are chromosome-based, showed diagnostic yields of 6.7 %, 12 %, and 13.3 %, respectively. In the NGS era, the diagnostic yield of WES using DEE was 66.7 % (Table 5).

Table 5. The diagnostic yields of genetic tests in the nationwide-Turkish EO-DEE cohort evaluated with a four-stepwise diagnostic model.

Gene-related DEEs: 589 / 1322 (44.5 %) The diagnostic rates with used genetic testing modalities		**Initial phase of NGS (2005–2013) *N* = 134/428 (31.3)**	**NGS era (2014–2021) *N* = 411/896 (45.8)**
Chromosome-based testing	Karyotype	4/111 (3.6)	22/273 (8)
	FISH	8/40 (20)	9/51 (17.6)
	Chromosomal microarray	5/60 (8.3)	20/127 (15.7)
NGS-based testing	Single gene analysis	40/89 (44.9)	60/153 (39.2)
	Epilepsy panels	34/71 (47.8)	85/214 (39.7)
	Whole exome sequencing	42/64 (65.6)	209/313(66.7)
	Mitochondrial panel	1/2 (50)	6/12 (50)

FISH: Fluorescent in situ hybridization, NGS: Next generation sequencing.

3.5. Cost-effectiveness of a stepwise diagnostic model

The cost-effectiveness of the stepwise diagnostic model was tested using the entire genetic testing cohort. A similar cost-effectiveness was defined for chromosome-based tests with respect to testing periods (initial phase of NGS versus the NGS era). However, the model indicated a higher overall cost for EO-DEEs in the NGS era (Table 6).

Table 6. The cost-effectiveness analysis of a four-stepwise diagnostic approach in the nationwide-Turkish EO-DEE cohort with EO-DEEs evaluated with a four-stepwise diagnostic model.

Test Modalities n (%)		**Initial phase of NGS *N* = 428 (32.3)**	**NGS era *N* = 896 (67.7)**	**p**
Chromosome-based tests	with stepwise *n* = 81 (15.8)	142±29 €	124±30 €	0.011
	non-stepwise *N* = 432 (84.2)	61±35 €	64±34 €	*0.117*

Test Modalities n (%)		Initial phase of NGS *N* = 428 (32.3)	NGS era *N* = 896 (67.7)	p
	p	<0.001	<0.001	
NGS-based tests	with stepwise *n* = 82 (16)	820±211 €	865±205 €	*0.199*
	non-stepwise *n* = 1165(88.4)	213±203 €	336±238 €	*<0.001*
	p	<0.001	<0.001	
Combined use of chromosome and NGS- based tests	with stepwise *n* = 137 (26.7)	3007±1496 €	3432±1359 €	*0.027*
	non-stepwise *n* = 376 (73.3)	741±690 €	1429±1188 €	*<0.001*
	p	<0.001	<0.001	

€ Euro.

4. Discussion

Persistent seizures, developmental delay or regression, and poor outcomes are all hallmarks of DEEs. Clarification of an etiology-specific diagnosis, including the identification of structural, metabolic, immune-infectious, or genetic causes, can impact treatment decisions for patients with DEE. Etiology-specific diagnoses have been reported to be achieved in 80 % of DEEs in the modern era using metabolomics, genomics, and digital imaging with new neuroradiologic modalities [[13], [14], [15]]. In this nationwide cohort study, genetic testing including the NGS technology and chromosome-based tests provided the rate of genetic etiology-specific diagnosis with a rate of 45.6 % in the whole cohort (initial phase of NGS and NGS era). NGS-based testing provided gene-related EO-DEEs in the two study periods: non-structural genetic (439, 62.6 %), metabolic-genetic (106, 15.1 %), and structural-genetic (99, 14.1 %). The most common nonstructural variants were *SCN1A* (16.9 %), *CDKL5* (3.7 %), *STXBP1* (2.4 %), *KCNQ2* (2.4 %), and *PCDH19* (2.2 %). Genetic testing enhanced the potential therapeutic yields of precision (33.6 %) and empirical therapies (41.3 %). The implications of precision therapy increased from 39 % in the initial phase of NGS to 50 % in the NGS era. A four-stepwise diagnostic model provided an etiology-specific diagnosis at a rate of 78.7 %.

To date, almost 400 genes related to epilepsy have been discovered, and the most commonly identified gene variants have been employed in exome sequencing and gene panels [[16], [17], [18]]. In this cohort study, NGS modalities yielded a higher number of gene-related EO-DEEs, with the genetic-non-structural, genetic-structural, and genetic-metabolic showing frequencies of 62.2 %, 14.5 %, and 14.8 %, respectively. The 12 most common variants in the genetic-nonstructural etiology subgroup (*SCN1A, STXBP1, CDKL5, KCNQ2, PCDH19, SCN8A, PRUNE1, CACNA1A, KCNT1, KCTD7, CHD2* and *MECP2*) accounted for 35.8 % of the gene-named DEEs. The spectrum of rare variants (detected at a rate of 0.5–1 %) in the initial phase of NGS (*n* = 7, *PRUNE1, KCTD7, CACNA1A, KCNT1, MECP2, SCN2A,*

CHD2) changed during the NGS era (*n* = 10, *SCN8A, KCTD7, CACNA1A, KCNT1, MECP2, SCN2A, CHD2, ALDH7A1, CLDC, SLC2A1*). The rate of ultra-rare (<0.5 %) variants increased in the NGS era (55 %) compared to the initial phase of NGS (37 %). A growing trend in genetic testing to identify the underlying genetic causes for patients who have acquired structural etiology-specific diagnoses has been observed, particularly among those with cerebral palsy-like disorders and congenital structural brain abnormalities. The cohort was defined using a distinct genetic-structural etiologic subgroup. The current study showed a notable increase in the predisposition towards genetic testing in subgroups characterized by unknown and structural etiologies with malformations of congenital disorders in the NGS era. In line with our findings, a recent multicenter study on infantile DEE, as well as an East Asian pediatric epilepsy cohort study reported that the most prominent category of single-gene variations consisted of nonstructural functional genes (e.g., *SCN1A, KNCQ2, SCN8A, SCN2A, STXBP1* and *CDKL5*) [8,19].

Prior research on DEEs has indicated that the presence of substantial variants in genes contributes to targeted therapies, with efficacies ranging from 17 % to 50 % [20,21]. The diagnosis influences the selection of the ASM, which can improve long-term prognosis and reduce mortality risk by enhancing seizure control. Furthermore, it permits the discontinuation of ASMs such as carbamazepine, which could exacerbate seizures in patients with Dravet syndrome. Scheffer et al. [18] previously reported that genetic diagnosis frequently led to management modifications in nearly 40 % of patients in whom the etiology could be determined. In the present nationwide cohort, genetic testing demonstrated potential therapeutic yields of 34.5 % and 56.2 % with precision and empirical therapy, respectively, in the cohort under investigation. The results of our study are consistent with those of previous reports showing that the treatment of 45 % of children with epilepsy is influenced in a variety of ways. These methods included a discussion of gene-specific clinical trials (10 %), the utilization of disease-specific vitamins or metabolic treatments (7 %), pathway-driven off-label use of medications (3 %), and the choice of ASM (36 %) [13,22,23].

In this cohort investigation, we established a slightly higher rate of etiology-specific diagnosis (78.7 %), which encompassed neuroimaging studies, comprehensive metabolic workup, and genetic testing. The incidence of unknown etiology decreased from 29.2 % (initial phase of NGS I-II, 2005–2009) to 18.7 % (NGS era, 2018–2021) across the different eras. This may be due to the increased utility of genetic testing in the cohort, which increased from 39.9 % in the initial phase of NGS to 66 % in the NGS era. However, the etiology was still unknown in 21.3 % of the entire cohort and 18.5 % of patients in the NGS era. For patients with uncertain genetic etiology, there is an urgent need to implement reverse genotyping with the reuse of current genetic testing modalities (WES, trio-based WES, chromosomal microarray, and mitochondrial gene panels) and new advanced modalities (WGS, high-resolution chromosomal microarray, RNA-based studies, and epigenetic studies) for each patient with negative results in the first three steps.

The diagnostic yield of genetic testing for DEEs has been reported to be between 10 % and 60 % when using NGS-based epilepsy panels and WES analysis [4,15,24]. The diagnostic yield of epilepsy panels is contingent on the incorporation of the gene spectrum. In this study, we defined the diagnostic yield of genetic testing using a four-stepwise diagnostic model. Genetic testing has provided etiology-specific diagnosis since the introduction of NGS in a stepwise diagnostic model in 2014. In the new genomic era (2014–2021), the diagnostic yields of the two most commonly used tests for DEEs are epilepsy panels (39.7 %) and WES (66.7 %). The diagnostic yields of epilepsy panels and WES

were comparable during the initial phases of the NGS and NGS eras. Genetic testing with comprehensive epilepsy panels is generally considered the standard medical practice for neonates with DEEs in the NGS era. WES exhibited a considerably higher diagnostic yield (66.1 %) than other genetic testing methods, comparable to that previously found in a Chinese cohort [13,23]. WES is a cost-effective method that can identify diverse genes in unexplained cases, and has the advantage of being reanalyzed in the future [13,25]. Furthermore, chromosomal microarrays can be used to identify diverse chromosomal abnormalities and copy number variants associated with DEEs [[26], [27], [28]]. Finally, the early application of genetic testing with a stepwise diagnostic model can be cost-effective, as an accurate diagnosis can prevent the need for superfluous and extensive diagnostic procedures for genetic etiology-specific diagnosis.

Epilepsy panels and chromosomal microarrays are effective methods for analyzing EO-DEEs during the initial phase of etiological diagnosis. Nevertheless, the notion of unknown “candidate genes" as the underlying cause of EO-DEEs has increased with new NGS technologies. Furthermore, epilepsy panels exhibit variability in terms of the number of genes examined and the clinical classification of patients, with the number of genes ranging from 12 to over 400, depending on the commercial business employed. The rates of ultra-rare variants of EO-DEEs have increased in the NGS era by up to 52 % following the extensive use of genetic tests. Phenotypic similarities among patients with early onset DEEs may lead to diverse success rates in identifying solutions. Wirrell et al. [23] recommended chromosomal microarray or epilepsy panels and basal metabolic testing as the initial diagnostic steps for patients with infantile epileptic spasm syndrome. If the initial test results were normal, WES was recommended as a cost-effective strategy. In subsequent years, new proposals emerged, indicating that conducting WES as an initial test would be more cost-effective and yield results more quickly.

The integration of WES into routine clinical practice represents a revolutionary shift for patients and their families, bringing an end to a protracted diagnostic odyssey that had previously caused considerable distress and frustration for families. Joshi et al. [29]. defined the time spent on a diagnostic journey as 1.4–8.2 years. The total cost of these traditional tests was higher at $9.015–$35.483 than that of WES ($ 6100). Therefore, the authors claimed that it is more cost-effective to prioritize WES over other tests, such as basic metabolic workup, chromosomal microarray, and epilepsy panels, when evaluating the impact of early onset DEEs on closure, future development, and genetic counseling. Two clinical recommendations for genetic testing in pediatric epilepsy have recently been outlined in these guidelines, as follows: (1) It is highly advisable that children of all ages undergo genetic testing for unexplained epilepsy. For initial testing, it is preferable to apply WES/WGS or epilepsy panels that include more than 25 genes, rather than a chromosomal microarray. Exome and genome sequencing is recommended for epilepsy panels. (2) In regards to genetic counseling before and after genetic tests, skilled healthcare providers must oversee the process of selecting, ordering, and interpreting tests [30]. Genetic testing with comprehensive epilepsy panels is generally considered the standard medical practice for infants with DEEs in the NGS era, but remains subject to availability. WES is a cost-effective method with the potential to identify numerous clinically relevant genes in enigmatic cases and provides the advantage of future re-examination [13,25]. Finally, the cost-effective potential of early genetic testing with a stepwise diagnostic model is evident, as a precise diagnosis can prevent the need for unnecessary and protracted diagnostic procedures for genetic etiology-specific diagnosis.

The objective of this cohort study conducted across the nation was to establish gene-named DEEs in terms of their therapeutic implications for targeted therapy, and to assess the etiologic trend of early onset DEEs using a cost-effective and stepwise diagnostic approach. Some limitations were introduced by the retrospective nature of this multicenter study. The lack of uniformity in the implementation of the stepwise approach across all institutions further imposed constraints on data collection. Nevertheless, most patients underwent MRI and basic metabolic screening, whereas etiology-specific diagnosis was administered in a stepwise diagnostic model, with a variety of genetic tests conducted at varying rates. The diagnostic ability was also enhanced by NGS. The diagnostic yield was highest for chromosomal microarrays, epilepsy panels, and WES. In this cohort, the novel etiological diversity of EO-DEEs with rare and ultra-rare variants was demonstrated with an expanding genetic landscape of EO-DEEs. In addition, the present study distinguishes itself from previous research by incorporating a temporal comparison to evaluate the etiological trends and efficacy of genetic testing in the genomic era (the initial phase of NGS versus the NGS era). Genetic reanalysis with additional in-depth or reverse phenotyping remains a valuable diagnostic tool to identify novel variants of uncertain significance in undiagnosed patients. After initial clinical evaluation and neuroimaging, a cost-effective genetic workup should be developed for patients lacking a specific etiology.

5. Conclusion

Genetic-etiology-specific diagnoses were further characterized for EO-DEEs to facilitate the management of patients undergoing antiseizure drug modification/precision therapy. However, a significant number of patients with unknown etiologies have a genetic etiology-specific diagnosis. We believe that reverse phenotyping using WES/WGS, epigenetic studies, and non-coding variant analysis should be performed for these EO-DEEs.

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