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Genotype-driven therapeutics in DEE and metabolic epilepsy: navigating treatment efficacy and drug resistance

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Neonatal intensive care unit (NICU), particularly in treating developmental and epileptic encephalopathy (DEE) and metabolic epilepsy (ME), requires a deep understanding of their complex etiologies and treatment responses. After excluding treatable cases such as infectious or autoimmune encephalitis, our focus shifted to a more challenging subgroup of 59 patients for in-depth genetic analysis using exome sequencing (ES). The ES analysis identified 40 genetic abnormalities, significantly including de novo variants. Notably, we found structural variation as duplications in regions 2q24.3, including *SCN1A* and *SCN2A* were observed in 7 cases. These genetic variants, impacting ion channels, glucose transport, transcription regulation, and kinases, play a crucial role in determining medication efficacy. More than one-third (34.2%) of patients with DEE had an unfavorable response to anti-seizure medications (ASMs) in the chronic phase. However, since the ketogenic supplementary diet showed a positive effect, more than three-quarters (80%) of these drug-resistant patients improved during a 3-month follow-up. In contrast, the ME had a lower adverse reaction rate of 9.1% (2/22) to specialized medications, yet there were 5 fatalities and 10 cases with unidentified genetic etiologies. This study suggests the potential of categorizing drug-resistant variants and that a ketogenic diet could be beneficial in managing DEE and ME. It also opens new perspectives on the mechanisms of the ketogenic diet on the discovered genetic variants.

Keywords Clinical sequencing, DEE, Metabolic epilepsy, Drug response, Ketogenic diet

Neonatal intensive care unit (NICU) may experience seizures due to a wide range of etiologies, including acute brain injury[1], stroke[2] and encephalitis[3,4], metabolic disturbances[5] and rare developmental and epileptic encephalopathy (DEE)[6]. Accurate identification and targeted management of the underlying causes of these seizures are paramount in conjuntion with seizure control itself[7]. The selection of appropriate anti-seizure medications (ASMs) presents a complex challenge, particularly in cases of DEE, where seizures frequency exhibit drug resistance[8]. This resistance necessitates compels the adoption of more innovative therapeutic approaches, such as the ketogenic diet (KD)[9], cannabidiol[10], and cranial epilepsy neurosurgery[11,12]. Effective management of these complex cases often demands a collaborative, multidisciplinary approach[13].

The integration of exome sequencing (ES) in the NICU practices marks a transformative shift in diagnostic methods, enabling a more profound understanding of the genetic factors driving these diseases[5]. Reflecting on our prior research on a DEE cohort from Vietnam, distinct genotype–phenotype correlations emerged, offering insights into potential therapeutic avenues[14]. Nevertheless, gaps persist in our comprehension of

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variant-specific therapeutic responses and the influence of metabolic anomalies on clinical presentations within DEE cohorts. Notably, as highlighted by Sadleir, even two variants within the same gene can lead to varied clinical manifestations[15], emphasizing the intricate interplay of functional dynamics in disease presentations and therapeutic complexities[16,17]. This challenge is further compounded by the fact that approximately one-third of patients do not respond optimally to ASMs, underscoring the urgency of refined diagnostic precision. In this evolving landscape, recent findings spotlighting genes such as *CDKL5*, *KCNT1*, *PCDH19*, *SCN1A*, *SCN2A*, *SCN8A*, *SLC2A1*, and *STXBP1* hold promise, suggesting that tailored therapeutic interventions based on specific genetic variants are useful[16,18]. Nevertheless, the sporadic nature of genotype-phenotype correlations complicates the ability to predict therapeutic outcomes solely on genetic data. Expanding our understanding of ion channels, kinases, and transcription factors is crucial to unraveling the molecular complexities inherent in these neurological conditions. Despite the potential of ES in the neurological NICU, its full implications remain underexplored, necessitating greater collaboration between clinicians and geneticists to fully harness its benefits.

This study aims to investigate the role of ES in the neonatal neurological NICU, with a particular focus on its impact on the diagnosis and management of DEE and metabolic epilepsy (ME). Furthermore, it seeks to address the challenges associated with ES, from the interpretation of results to the management of incidental findings, with the overarching goal of optimizing patient outcomes.

Results

Patient distribution and treatment response in the NICU

A comprehensive review was conducted on 172 NICU patients, as outlined in the flow chart presented in Fig. 1, focusing on risk classification. In this group, most patients (113 in total) showed improvement and successfully controlled seizures caused by infectious or autoimmune encephalitis, all within seven days of initiating first-line treatment. These treatable patients were subsequently excluded from the genetic study group. Additionally, a subset of 59 patients with an average seizure age of onset of 2 years and 6 months, accounting for 34.3% of the total patients, were subjected to ES analysis and received ongoing monitoring via pediatric neurology. In this subset, the preliminary MRI diagnosis revealed specific abnormalities, such as hypoplasia of the corpus callosum, diffuse cortical dysplasia, polymicrogyria, focal cortical dysplasia, hippocampal sclerosis, corpus callosum agenesis, mild cortical atrophy, cortical dysplasia, generalized brain atrophy, cortical malformation, periventricular leukomalacia, and cases categorized as uncertain. The phenotypic breakdowns included likely Lennox-Gastaut syndrome (MIM#301058, 7 patients), Dravet syndrome (MIM#607208, 8 patients), Early infantile developmental and epileptic encephalopathy (EIDEE) previously known as Ohtahara syndrome (MIM#308350, 8 patients), Infantile epileptic spasm syndrome (IESS) or previously known as West syndrome (MIM#308350, 9 patients), and undefined phenotypes (5 patients) (Table 1). Moreover, a significant segment of this group ($n=22$) was identified with ME, characterized by abnormal blood and CSF profiles; elevated lactate, ammonia, or atypical amino acid levels; and MRI evidence of cerebral atrophy or white matter anomalies (Table 2). EEG findings, while indicative of the respective diagnostic categories, presented overlapping features across different patients (Tables 1 and 2).

Genetic variants in DEE

Three weeks after initiating exome sequencing, 17 genetic anomalies were identified, spanning the DEE spectrum. These variations predominantly included missense variants, with the rest being nonsense and frameshift variants, as illustrated in Fig. 2 and Table S1, and their protein domain and variation location are shown in Figure S1a–j. Notably, seven patients exhibited duplications at 2q24.3 regions, which are associated with the *SCN1A* and *SCN2A* regions, respectively (Figure S3a–b). In the DEE group, the variations spanned ten genes and fell into three functional categories: ion channels (75.7%), regulators (18.9%), and kinases (5.4%). A significant 93.5% of these variants were *de novo* variants, indicating sporadic occurrence. Of these variants, 87.1% were predicted to alter protein function, while the rest were protein truncating (12.9%). The channelopathies subgroup included 21 *de novo* variants across multiple genes, mostly missense variants (Tables S1 and Figure S2). Following the criteria established by the ACMG classification, we categorized these variants into distinct levels of pathogenicity, namely, pathogenic, likely pathogenic, variant of uncertain significance (VUS), and likely benign, as detailed in Table S1. The regulatory and kinase group included variants in the *STXBP1*, *ARX*, and *CDKL5*, featuring a mix of protein-truncating variants (PTVs) and protein-alternating variants (PAVs).

Genetic variants in the ME group

In the ME group, 45.5% of patients had no detectable genetic etiology. However, exome sequencing uncovered seven implicated genes in 54.5% of patients, revealing 18 genetic variants (Table S1) along with their respective protein domain and variation locations, as illustrated in Figure S1k–p. These were classified as likely pathogenic, pathogenic, or VUS, with in silico scores and inheritance patterns (*de novo*, paternal, maternal) detailed in Tables S1 and Figure S2.

Associations between genotype and ASMs

In the DEE group, we identified 12 genes associated with variable clinical presentations. Four ion channel-related variants from *GABRB3*, *SCN1A*, and *SCN2A* showed a positive response to prolonged treatment, while the remaining eight variants did not respond. Conversely, *ARX* variants, particularly in three male patients exhibiting an EEG pattern of burst suppression, were linked to ongoing seizures despite treatment with valproic acid (VPA), cannabidiol (CBD), and adrenocorticotropic hormone (ACTH). *CDKL5* was noted in two patients with a phenotypic diagnosis of EIDEE; these patients were both resistant to phenobarbital (PHB) and ACTH. Variant in *KCNQ2* such as c.749T > C; (p.Val250Ala) was identified in patients with Early infantile developmental and epileptic encephalopathies (EIDEE) who did not respond to combined VPA, lamotrigine (LMG),

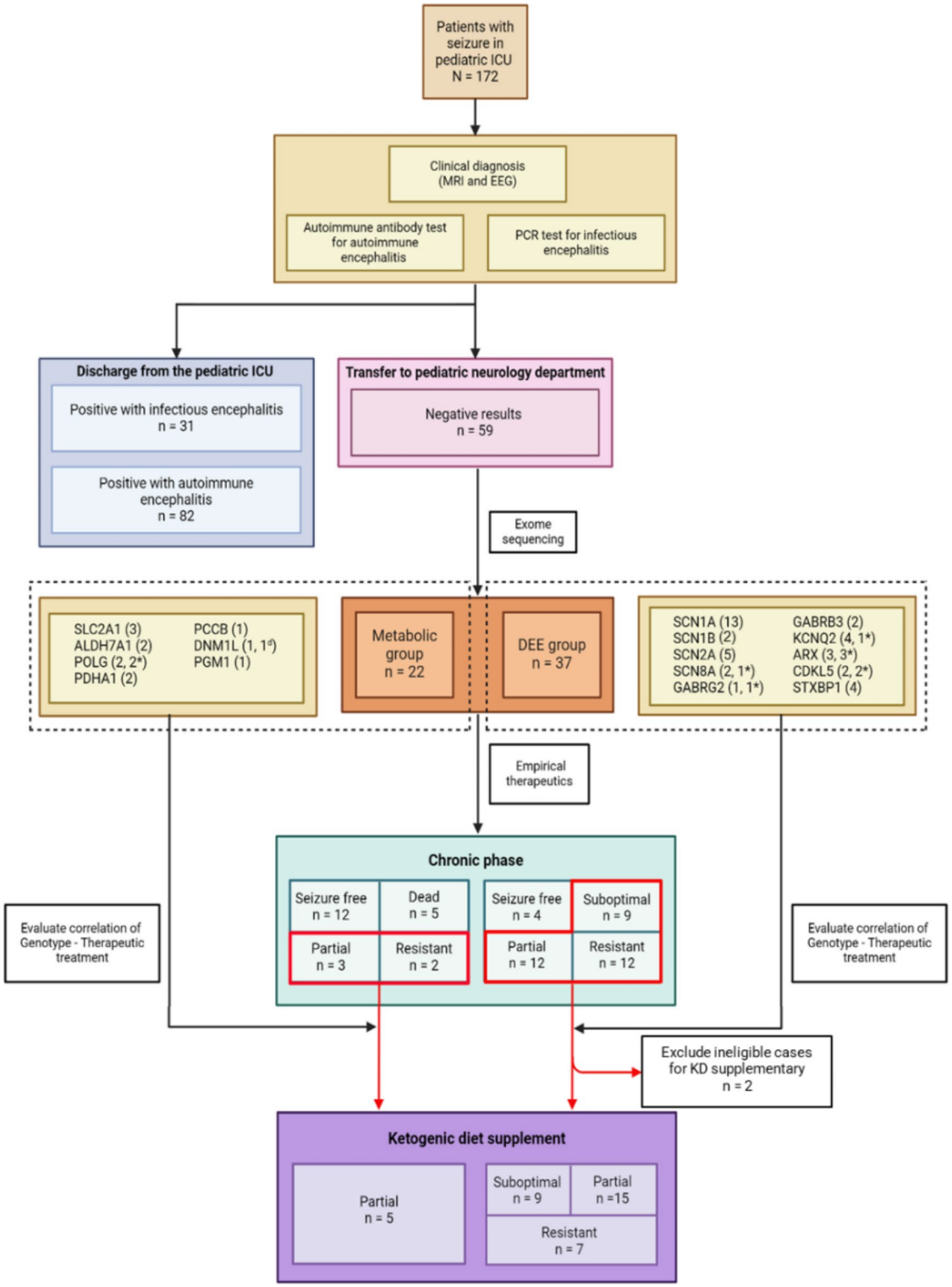

Fig. 1. Flowchart outlining the study of pediatric NICU patients with seizures. A total of 172 patients underwent tests for autoimmune and infectious encephalitis. Positive patients were treated accordingly, while those testing negative were referred for genetic analysis via exome sequencing (ES), splitting them into ME and DEE groups. The results of ES are briefly represented as gene names with accompanying numbers in the following order: the first number indicates variants in drug-responsive cases, the second number specifies variants marked with an asterisk (*) for therapeutic resistance or 'd' for decreased response situations. Long-term outcomes, including seizure control, resistance, and mortality, were monitored. Some patients receive a KD as a supplement, especially those with a poor response to ASMs. Treatment success was assessed in relation to the patients' genetic profiles.

ID	A/S	Genetic variant	Inheritance	Reported/ Novel	Phenoty-pe	Drug Response	Treatment	MRI findings	EEG findings
1	3/M	*SCN1A*(NM_001165963.4):c.4942 C > T p.(Arg1648Cys)	*De novo*	Reported[51]	DS	Suboptimal	VPA, CBD	Mild cortical atrophy	Increased spike-wave activity
2	1/F	*SCN1B*(NM_001037.5):c.254_255delGCinsAA p.(Arg85Gln) *SCN1B*(NM_001037.5):c.457G > A p.(Asp153Asn)	Paternal Maternal	Novel Reported[52]	ND	Suboptimal	VPA, CBD	Generalized brain atrophy	Burst suppression pattern
3	2/M	*STXBP1*(NM_003165.6):c.589G > C p.(Asp197His)	*De novo*	Novel	ND	Partial	VPA	Uncertain	Focal epileptiform discharges
4	2/M	*CDKL5*(NM_001323289.2):c.2341_2342delAGinsTA p.(Arg781Ter)	*De novo*	Novel	EIDEE	Resistant	PHB, ACTH	Diffuse cortical dysplasia	Normal
5	3/M	*SCN1A*(NM_001165963.4):c.3976G > T p.(Ala1326Ser)	*De novo*	Novel	DS	Partial	VPA, CBD	Normal	Focal epileptiform discharges
6	3/F	*KCNQ2*(NM_172107.4):c.1259 C > T p.(Pro420Leu)	*De novo*	Novel	LGS	Suboptimal	VPA, LMG, TPM, CBD	Normal	Normal
7	3/M	*SCN2A*(NM_001040143.2):c.2557 C > T p.(Arg853Trp)	*De novo*	Novel	IESS	Suboptimal	ACTH, VGB, TPM	Cortical malformation	Increased spike-wave activity
8	3/M	*ARX*(NM_139058.3):c.1112G > A p.(Arg371Gln)	*De novo*	Reported[53]	ND	Resistant	VPA, CBD	Hypoplasia of corpus callosum	Burst suppression pattern
9	4/M	*SCN1A*(NM_001165963.4):c.2191_2192delAGinsCA p.(Arg731Gln)	*De novo*	Novel	DS	Partial	VPA, CBD	Normal	Normal
10	4/F	*GABRB3*(NM_000814.6):c.613 A > G (p.Arg205Gly)	*De novo*	Novel	LGS	Complete	VPA, LMG, TPM, CBD	Normal	Normal
11	4/F	*STXBP1*(NM_003165.6):c.728T > G p.(Val243Gly)	*De novo*	Novel	IESS	Partial	VGB, TPM	Periventricular leukomalacia	Hypsarrhythmia pattern
12	5/F	*SCN8A*(NM_014191.4):c.2803_2804delGGinsTC p.(Gly935Ser)	*De novo*	Novel	ND	Resistant	VPA, CBD	Normal	Generalized spike-wave activity
13	3/M	*SCN1A*(NM_001165963.4):c.559 C > T p.(Arg187Trp)	*De novo*	Novel	DS	Suboptimal	VPA, CBD	Cortical dysplasia	Normal
14	3/M	*KCNQ2*(NM_172107.4):c.749T > C p.(Val250Ala)	*De novo*	Novel	EIDEE	Resistant	VPA, LMG, TPM, CBD	Corpus callosum agenesis	Burst suppression pattern
15	2/F	*SCN2A*(NM_001040143.2):c.400 C > T p.(Leu134Phe)	*De novo*	Novel	IESS	Complete	ACTH, VGB, TPM	Uncertain	Increased spike-wave activity
16	5/F	*CDKL5*(NM_001323289.2):c.2257 C > T (p.Gln753Ter)	*De novo*	Novel	EIDEE	Resistant	PHB, ACTH	Polymicrogyria	Normal
17	2/F	*SCN1A*(NM_001165963.4):c.4408_4409delGGinsTC p.(Gly1470Ser)	*De novo*	Novel	DS	Partial	VPA, CBD	Normal	Focal epileptiform discharges
18	5/M	*GABRG2*(NM_198904.4):c.644_645delGTinsAG p.(Arg215Gln)	*De novo*	Novel	LGS	Resistant	VPA, LMG, TPM, CBD	Normal	Generalized slow waves
19	1/M	*STXBP1*(NM_003165.6):c.1450G > C p.(Asp484His)	*De novo*	Novel	IESS	Partial	VGB, TPM	Cortical malformation	Hypsarrhythmia pattern
20	2/M	*ARX*(NM_139058.3):c.736_737delGAinsTG p.(Asp246Cys)	*De novo*	Novel	EIDEE	Resistant	PHB, ACTH	Normal	Burst suppression pattern
21	5/F	*SCN1A*(NM_001165963.4):c.3905 A > G p.(Asn1302Ser)	*De novo*	Novel	DS	Complete	STP, VPA, CBD	Normal	Normal
22	1/M	*KCNQ2*(NM_172107.4):c.637 C > G p.(Arg213Gly)	*De novo*	Novel	LGS	Suboptimal	VPA, LMG, TPM, CBD	Hippocampal sclerosis	Normal
23	2/M	*SCN2A*(NM_001040143.2):c.2567G > A p.(Arg856Gln)	*De novo*	Reported[54]	IESS	Partial	ACTH, VGB, TPM	Cortical malformation	Increased spike-wave activity
24	3/M	*ARX*(NM_139058.3):c.1096_1098delGACinsTAA p.(Asp366Ter)	*De novo*	Novel	EIDEE	Resistant	PHB, ACTH	Normal	Burst suppression pattern
25	5/M	*SCN1A*(NM_001165963.4):c.2135G > A p.(Arg712Gln)	*De novo*	Novel	DS	Suboptimal	VPA, CBD	Normal	Normal
26	4/F	*GABRB3*(NM_000814.6):c.580 C > G p.(Arg194Gly)	*De novo*	Novel	LGS	Partial	VPA, LMG, TPM, CBD	Focal cortical dysplasia	Generalized slow waves
27	5/M	*STXBP1*(NM_003165.6):c.703 C > T p.(Arg235Ter)	*De novo*	Reported[55]	IESS	Partial	VGB, TPM	Periventricular leukomalacia	Hypsarrhythmia pattern
28	5/M	*SCN8A*(NM_014191.4):c.2746 C > T p.(Arg916Cys)	*De novo*	Novel	ND	Suboptimal	VPA, CBD	Normal	Normal
29	4/F	*SCN1A*(NM_001165963.4):c.574T > A p.(Trp192Arg)	*De novo*	Novel	DS	Complete	VPA, CBD	Cortical dysplasia	Focal epileptiform discharges

Continued

ID	A/S	Genetic variant	Inheritance	Reported/ Novel	Phenoty-pe	Drug Response	Treatment	MRI findings	EEG findings
30	4/M	*KCNQ2*(NM_172107.4):c.637 C > T p.(Arg213Trp)	*De novo*	Reported[56]	LGS	Suboptimal	VPA, LMG, TPM, CBD	Corpus callosum agenesis	Burst suppression pattern
31	1/F	*SCN1A*: 2q24.3 (chr2:165985813–166128020) x4	*De novo*	Novel	EIDEE	Partial	PHB, ACTH	Generalized brain atrophy	Focal epileptiform discharges
32	1/M	*SCN1A*: 2q24.3 (chr2:165985813–166128020) x4	*De novo*	Novel	IESS	Resistant	ACTH, VGB, TPM	Generalized brain atrophy	Focal epileptiform discharges
33	1/F	*SCN1A*: 2q24.3 (chr2:165985813–166128020) x4	*De novo*	Novel	IESS	Resistant	ACTH, VGB, TPM	Generalized brain atrophy	Focal epileptiform discharges
34	1/M	*SCN2A*: 2q24.3 (chr2:165294055–165392304) x3	*De novo*	Novel	IESS	Resistant	ACTH, VGB, TPM	Cortical dysplasia	Focal epileptiform discharges
35	1/F	*SCN2A*: 2q24.3 (chr2:165294055–165392304) x3	*De novo*	Novel	EIDEE	Partial	PHB, ACTH	Cortical dysplasia	Focal epileptiform discharges
36	1/F	*SCN1A*: 2q24.3 (chr2:165985813–166128020) x4	*De novo*	Novel	EIDEE	Partial	PHB, ACTH	Generalized brain atrophy	Focal epileptiform discharges
37	1/M	*SCN1A*: 2q24.3 (chr2:165985813–166128020) x4	*De novo*	Novel	EIDEE	Resistant	PHB, ACTH	Generalized brain atrophy	Focal epileptiform discharges

Table 1. Clinical feature of DEE group. *A/S*: Age/Sex; *F*: Female, *M*: Male; *CNV*: Copy number variation; *DS*: Dravet syndrome, *LGS*: Lennox-Gastaut syndrome, *EIDEE*: Early infantile developmental and epileptic encephalopathy, *IESS*: Infantile epileptic spasms syndrome; *STP*: Stiripentol, *VPA*: Valproate, *CBD*: Cannabidiol, *LMG*: Lamotrigine, *TPM*: Topiramate, *ACTH*: Adrenocorticotropic hormone, *VGB*: Vigabatrin, *PHB*: Phenobarbital, *CLZ*: Clonazepam.

topiramate (TPM), or CBD treatment. Patient #12, with the *SCN8A*: c.2803_2804delGGinsTC; (p.Gly935Ser) variant, showed no significant improvement in response to VPA or CBD. A *GABRG2*: c.644_645delGTinsAG; (p.Arg215Gln) was also linked to a lack of response to combined VPA, LMG, TPM, and CBD.

In the ME group, most patients experienced seizure reduction or complementation with ASMs combined with a ketogenic, low-protein diet or special supplements. Two female patients with 2-year-old onset of disease were detected with heterozygous altered variants in the *POLG* (one with c.1849_1851delCGTinsTGA; (p.Arg617Ter) and c.2740 A > C; (p.Thr914Pro); one with p.Lys1035SerfsTer59 and c.424_425delCTinsGC; (p.Leu142Ala)) (Fig. 3; Table 2). MRI revealed progressive cerebral atrophy and basal ganglia changes, and the patients developed drug resistance and Alpers – Huttenlocher syndrome. Despite a good initial response to ASM for seizure control, Patient #53 with nonsense variant of *DNM1L*: c.289 A > T; (p.Lys97Ter) exhibited persistent lactic acidemia in blood and cerebrospinal fluid (CSF), and nonspecific abnormalities in EEG and MRI ultimately indicated an intractable drug response, followed by death (Table 2).

Supplementation with a ketogenic diet

The three-month assessment of the KD included 36 patients from DEE and ME groups, encompassing 20 males and 16 females, who continued to experience seizures despite receiving multiple ASMs. Only 2 patients (patients #34 and #37) were deemed ineligible for KD supplementation due to nutritional inadequacies. The data revealed that, in the DEE group, 59.1% (13/22) of the epilepsy patients experienced a shift in their response patterns when the KD was combined with multiple ASMs. Specifically, the addition of the KD benefited 36.4% (8/22) of the individuals, transitioning them from a drug-resistant state to a partial response. In contrast, 22.7% (5/22) of the patients (two patients with missense variants and three patients with duplications in *SCN1A* and *SCN2A*) who had a partial response to ASMs became increasingly worse and more resistant to the KD supplement (Fig. 3; Table 1). Within the ME group, 40% (2/5) of patients with Alpers-Huttenlocher syndrome exhibited a change in response pattern, transitioning from resistance to multiple ASMs therapies to a partial response when a KD supplement was added to their treatment regimen (Fig. 3).

Discussion

These findings align with previous studies on genetic epilepsy, reinforcing the validity and prevalence of genetic markers across diverse populations[19]. This consistency provides a notable diagnostic yield, particularly in identifying novel disease variants, including those associated with actionable drug genes that are crucial for precision medicine. Notably, we found that approximately 43.75% of the candidate genes were associated with channelopathies—disorders caused by dysfunctional ion channels. *SCN1A* emerged as a recurrently implicated gene in DEEs across various cohorts, including ours, highlighting its critical role in both diagnosis and treatment[20–22] (Figure S3). Our study also highlighted the genetic heterogeneity underlying DEEs such as *SCN2A*, *SCN1B*, *SCN8A*, *GABRG2*, *GABRG3* and *KCNQ2* (variants are displayed in Figure S1 a—j). In addition, the findings revealed *ARX*, *CDKL5*, *STXBP1*, *ALDH7A1*, *POLG*, *PDHA1*, *PCCB*, *DNM1L*, and *PGM1* (variants

ID	A/S	Genetic variant	Inheritance	Reported/ Novel	Phenotype	Drug response	MRI Findings	EEG findings
38	3y/M	*SLC2A1*:c.679 + 5G > T	De novo	Novel	GLUT1-DS, Elevated lactate in blood and CSF	Complete	Generalized cerebral atrophy	Interictal diffuse slow background activity, intermittent focal or generalized slowing
39	3y/M	*SLC2A1*(NM_006516.4):c.399 C > A p.(Cys133Ter)	Maternal	Novel	GLUT1-DS, Elevated lactate in blood and CSF	Complete	Normal or non-specific abnormalities	Interictal diffuse slow background activity, intermittent focal or generalized slowing
40	3y/M	*ALDH7A1*(NM_001182.5):c.1375 A > T p.(Ile459Phe) *ALDH7A1*(NM_001182.5):c.608G > A p.(Trp203Ter)	Paternal Paternal	Novel Novel	PDE	Complete	Variable findings (cortical atrophy, atypical myelination, basal ganglia changes)	Burst-suppression pattern, multifocal epileptiform discharges
41	4y/F	*ALDH7A1*(NM_001182.5):c.1003 C > T (p.Arg335Ter) *ALDH7A1*(NM_001182.5):c.538dup (p.Glu180fsGlyfsTer48)	Paternal Paternal	Reported[57] Novel	PDE	Complete	Variable findings (cortical atrophy, atypical myelination, basal ganglia changes)	Burst-suppression pattern, multifocal epileptiform discharges
42	2y/F	*POLG*(NM_002693.3):c.1849_1851delCGTinsTGA p.(Arg617Ter) *POLG*(NM_002693.3):c.2740 A > C p.(Thr914Pro)	Paternal Paternal	Novel Reported[58]	AHS	Resistant	Progressive cerebral atrophy, basal ganglia changes	Slow background activity, multifocal or generalized epileptiform discharges
43	2y/F	*POLG*(NM_002693.3):c.3104dup p.(Ser1036ValfsTer11) *POLG*(NM_002693.3):c.424_425delCTinsGC p.(Leu142Ala)	Paternal Paternal	Novel Novel	AHS	Resistant	Progressive cerebral atrophy, basal ganglia changes	Slow background activity, multifocal or generalized epileptiform discharges
44	1y/M	*PDHA1*(NM_000284.4):c.685_686delATinsTA p.(Met229Ter)	Maternal	Novel	PDCD, Elevated lactate in blood and CSF	Complete	Normal or non-specific abnormalities	Interictal slowing, multifocal or generalized epileptiform discharges
45	2y/M	*PDHA1*(NM_000284.4):c.839T > G p.(Ile280Ser)	Maternal	Reported[59]	PDCD, Elevated lactate in blood and CSF	Complete	Normal or non-specific abnormalities	Interictal slowing, multifocal or generalized epileptiform discharges
46	2y/F	Non-detected genetic etiology	-	-	Epilepsy with low glucose in blood, Elevated lactate in blood and CSF	Partial	Normal or non-specific abnormalities	Normal or non-specific abnormalities
47	2y/F	Non-detected genetic etiology	-	-	Epilepsy with low glucose in blood, Elevated lactate in blood and CSF	Partial	Normal or non-specific abnormalities	Normal or non-specific abnormalities
48	2y/F	Non-detected genetic etiology	-	-	Propionic acidemia: Elevated levels of propionic acid, 3-hydroxypropionic acid, methylcitric acid	Partial	Normal or non-specific abnormalities	Normal or non-specific abnormalities
49	3y/F	Non-detected genetic etiology	-	-	Elevated levels of guanidinoacetate in blood or urine.	Complete	Variable findings (cortical atrophy, atypical myelination, basal ganglia changes)	Burst-suppression pattern, multifocal epileptiform discharges
50	2y/M	Non-detected genetic etiology	-	-	Glycosylation Disorders	Complete	Generalized cerebral atrophy	Normal or non-specific abnormalities
51	2y/M	Non-detected genetic etiology	-	-	Elevated ammonia in blood and/or urine, Elevated lactate in blood and CSF	Complete	White matter abnormalities	Normal or non-specific abnormalities
52	2y/M	*PCCB*(NM_000532.5):c.1126 C > T p.(Arg376Cys) *PCCB*(NM_000532.5):c.1531dup p.(Thr511AsnfsTer8)	Paternal Paternal	Reported[60] Novel	Propionic acidemia	Complete	Normal or non-specific abnormalities	Normal or non-specific abnormalities
53	1 m /M	*DNM1L*(NM_012062.5):c.289 A > T p.(Lys97Ter)	*De novo*	Novel	Persistent lactic acidemia in blood and CSF	Deceased	ND	ND
54	4y/F	*SLC2A1*(NM_006516.4):c.766 A > T p.(Lys256Ter)	*De novo*	Novel	Limb ataxia, GLUT1-DS	Complete	ND	Normal or non-specific abnormalities
55	2y/F	Non-detected genetic etiology	-	-	Persistent lactic acidemia in blood and CSF	Deceased	ND	ND

Continued

ID	A/S	Genetic variant	Inheritance	Reported/ Novel	Phenotype	Drug response	MRI Findings	EEG findings
56	6 m/F	Non-detected genetic etiology	-	-	Episodically elevated lactate in blood and CSF	Deceased	ND	ND
57	8 m/M	Non-detected genetic etiology	-	-	Persistent lactic acidemia in blood and CSF	Deceased	White matter abnormalities	ND
58	1y/F	Non-detected genetic etiology	-	-	Episodically elevated lactate in blood and CSF	Deceased	White matter abnormalities	Normal or non-specific abnormalities
59	2y6m/F	*PGM1*(NM_002633.3):c.43 C > T p.(Gln15Ter) *PGM1*(NM_002633.3):c.1324 A > G p.(Met442Val)	Paternal Paternal	Novel Novel	Congenital Disorder of Glycosylation, Type It	Complete	Normal or non-specific abnormalities	Normal or non-specific abnormalities

Table 2. Clinical feature of ME group. *A/S*: Age/Sex, y: year, m: month; *F*: Female, *M*: Male; *GLUT1-DS*: Glucose transporter type 1 deficiency syndrome, *PDE*: Pyridoxine-dependent epilepsy, *AHS*: Alpers-Huttenlocher syndrome, *PDCD*: Pyruvate dehydrogenase complex deficiency; *CSF*: cerebrospinal fluid, *ND*: Non-determined.

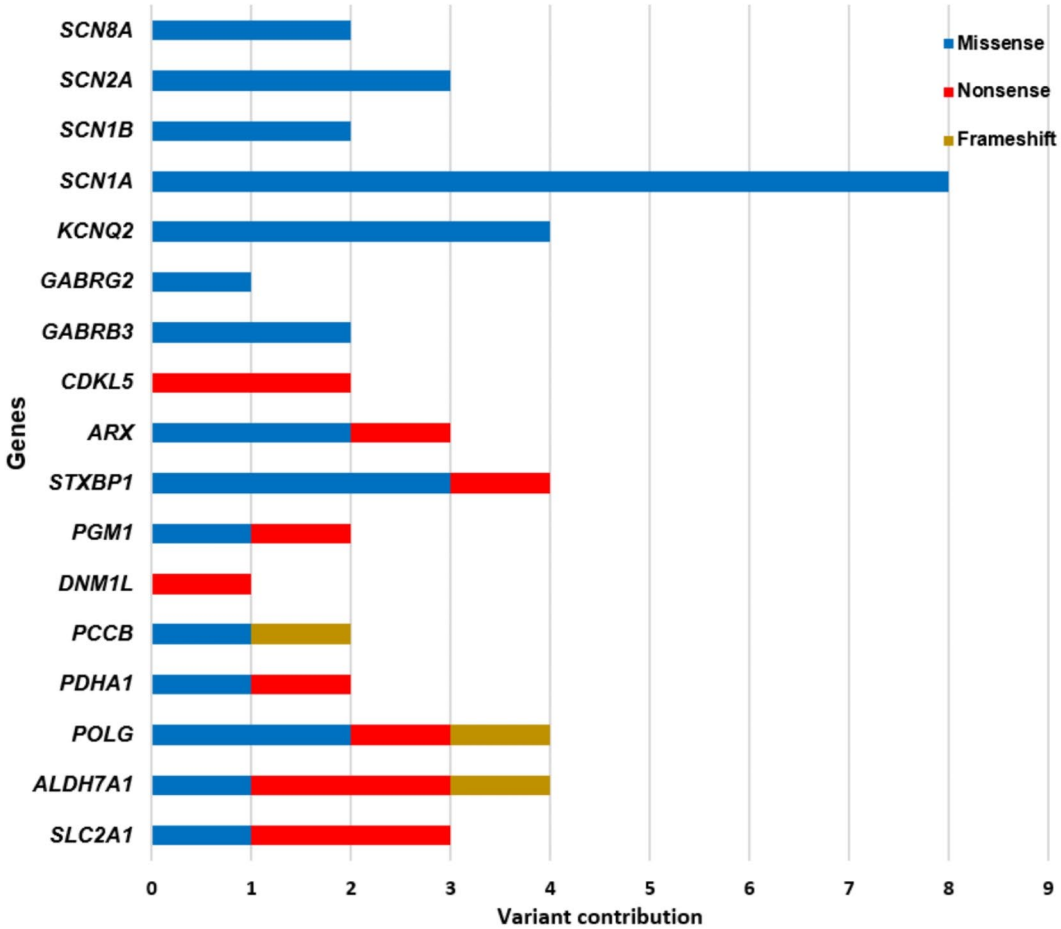

Fig. 2. Variant types across genes for DEE and ME group with *SCN1A* shows the highest number of missense variants, while *CDKL5* has a substantial number of both missense and nonsense variants. *PGM1* showing mixed missense/nonsense and *POLG* having a notable frameshift presence.

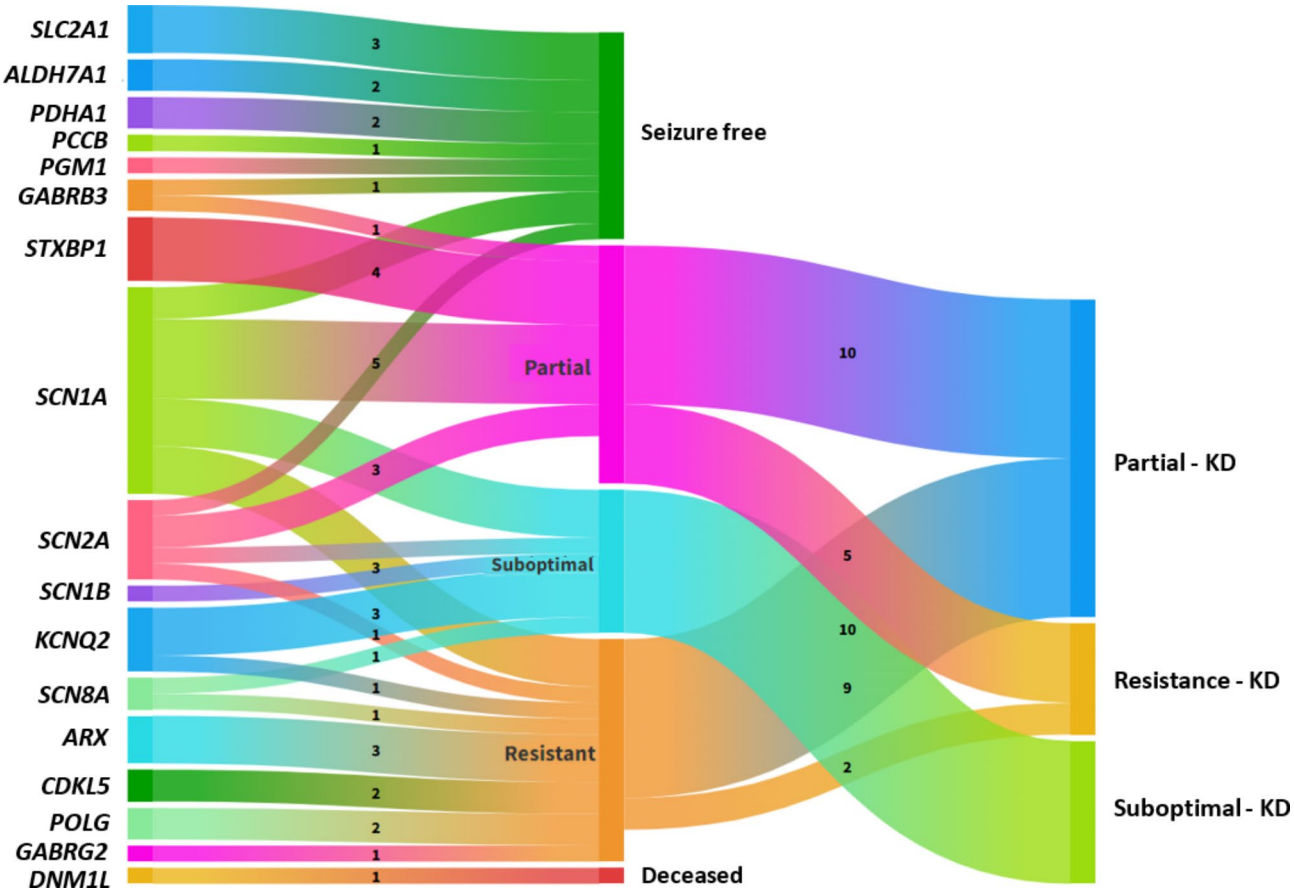

Fig. 3. Overall treatment response by genetic variation. The Sankey diagram maps genetic variants to treatment responses, including ASMs and a KD. The left panel lists genes associated with seizure conditions. The middle panel shows ASM response categories to treatments: seizure-free, partial, resistant, suboptimal, and dead. The right side shows how these responses might change with the addition of a KD, with categories such as partial – KD and resistant – KD, suggesting partial or full resistance to treatment despite the diet. The width of the bands represents the number of patients exhibiting each treatment response per genetic variant.

are displayed in Figure S1 k—p), suggesting the ongoing evolution of genomic knowledge and its impact on improving diagnostic precision.

Neonatal seizures present unique challenges, including variable causes, symptom severity, and patient responses. The current literature indicates the need for standardized criteria for evaluating antiseizure efficacy in newborns, complicating the optimization of treatment and dosage[8]. Yozawitz's study also mentioned that when conventional antiseizure therapy fails to be controlled, clinicians could consider that the patients had abnormal metabolic findings causing ME. This was also reflected in our genetic cohort, in which the ES revealed that 20.3% (12/59) of the genetic cohort carried a genetically metabolic etiology. Based on genetic etiology, we categorized individuals with ME for whom KD therapy has shown benefits. Our records indicate that most of our patients had a favorable response to the combined regimen of ASMs and additional interventions such as KD, D-galactose, specific dietary plans, or specialized supplements. Thus, genetic testing should be considered when a genetic cause is suspected, as it can enable precision therapy approaches. Some genes may be strongly associated with typical phenotypes, providing clues about the underlying cause and potential pathogenic variants.

We observed a patient with the substitution variant of *SCN8A*, c.2803_2804delGGinsTC; (p.Gly935Ser) in transmembrane segment 2 (Figure S1d) who was resistant to sodium channel blockers (SCBs). Although classified as a variant of uncertain significance, this variant had an intolerant impact (Figure S5a) on protein stability (-1.13 of DDG score). This was suspected to be the reason for resistance to VPA and CBD[23]. *SCN8A* variants have been linked to gain-of-function (GoF) and loss-of-function (LoF) epilepsies[24]. Determining the specific action of such variants through functional validation is crucial for precision medicine.

Within our cohort, pathogenic PAVs in *KCNQ2* exhibited a range of phenotypes. Most patients responded to SCBs (such as VPA, LMG, TPM, or CBD). However, one patient with the missense variant, c.749T > C; (p.Val250Ala) in *KCNQ2* was resistant, suggesting that the location and functional status of the variant significantly influenced the drug response (Figure S4b and Figure S5b). It is hypothesized that ASMs bind to the pore region of channels and that variants in this area could impair binding, necessitating functional analyses to inform treatment. Previous findings have shown that ASMs likely target the pore region of channels, suggesting that variants in this vicinity could interfere with ASM binding[25]. Interestingly, these findings include

a patient with a missense variant at the same Val250 position but different in altering amino acid, c.748G > C; (p.Val250Leu) who showed a positive response to ASMs, such as VPA and levetiracetam (LEV), suggesting these drugs for individuals with *KCNQ2* variants[25]. The variability in treatment outcomes may be attributed to the specific functional implications of the variants. Notably, our finding that Val250Ala leads to a considerable reduction in protein stability (a DDG score of -1.87) contradicts that of Val250Leu, which causes only a minor decrease (DDG score of -0.57). The resistance observed with Val250Ala in response to SCBs raises suspicions of a gain-of-function phenotype in *KCNQ2*. Consequently, functional assays are advocated for exploring the disease mechanisms of Kv7.2 channels in the context of drug interactions.

GABRG2 encodes for heterotetrametric *GABAA* receptors consisting of extracellular N-terminal and transmembrane domains also shows variability in drug response. The patient with substitution variant, c.644_645delGTinsAG; (p.Arg215Gln) in *GABRG2* did not respond to standard medication, supporting the hypothesis that novel variants can disrupt protein structure, affecting receptors[26]. At amino acid 215 (Figure S1f), the positively charged arginine was altered to neutrally charged glutamine, resulting in an unstable protein (-1.11 for the DDG score) (Figure S4c). As a result, we hypothesize that novel variants can disrupt the structural domain, affect surface tracking, and cause concentration of the subunit in the endoplasmic reticulum[26,27]. In addition, several variants, namely, Ala118Cys, Arg177Gly, and Ile218Ser, which are also located in the extracellular N-terminal domain, respond well to ASM, whereas Pro83Ser, Ile107Ter, and Ala106Ter exhibit drug resistance and penetrance[27]. Variants in the extracellular N-terminal domain usually respond well to ASM, while variants leading to resistance highlight the complexity of predicting and selecting appropriate therapies.

Previous reports have indicated that *CDKL5* variants have been commonly found in females with early-onset seizures and is involved in DEE and WS[28]. The X-linked inheritance pattern has led to fewer reported instances of male carriers of *CDKL5* variants, with some cases hinting at early male mortality. Our study revealed two unrelated pediatric cases of *CDKL5*-related diseases in which the nonsense mutations, c.2341_2342delAGinsTA; (p.Arg781Ter) and c.2257 C > T; (p.Gln753Ter) changed the structure and subsequently affected protein function (Figure S1i and Figure S4d). These patients resisted PHB and ACTH therapy, as did patients with severe anomalies in EEG and neuroimaging findings. This finding supports the notion that *CDKL5* is a significant genetic contributor to neonatal seizures and profound developmental challenges. Consistent with the literature, *CDKL5* variants tend to result in severe clinical manifestations in both sexes. Nonetheless, the severity of symptoms can vary by sex, as observed in a family with fraternal twins in which the male was more severely affected than the female was[29]. Additional reports corroborate this sex disparity, describing a cohort of males with *CDKL5* variants who experienced refractory epilepsy, significant developmental delays, and adverse neurocognitive outcomes[30].

ARX, which is vital for neuroblast proliferation and GABAergic neuron migration[31], is linked to various phenotypes and often confers resistance to ASMs. These include X-linked West syndrome, X-linked myoclonic epilepsy with developmental dysplasia, Partington syndrome, and X-linked lissencephaly with ambiguous genitalia (XLAG)[32]. Additionally, brain malformations such as lissencephaly, agenesis of the corpus callosum, and midbrain malformations are commonly observed in male *ARX* individuals[32]. Our study identified three distinct *ARX* variants in male patients across two domains: in the homeodomain, a missense variant c.1112G > A; (p.Arg371Gln) and a nonsense variant c.1096_1098delGACinsTAA; (p.Asp366Ter); and in the acidic domain, a missense variant c.736_737delGAinsTG; (p.Asp246Cys) (Figure S1h). These variants, located in highly conserved areas, have an intolerant impact and may lead to abnormal protein function affecting DNA binding (Figure S4e and Figure S5d). This finding suggested that male patients with missense or nonsense variants in the homeodomain could have severe brain malformations and cognitive impairments and may respond poorly to drug treatments.

Copy number variants are known as a significantly recognized factor in the genetics of epilepsy. Through multiple large – scale studies, genomic "hotpots" associated with high-risk epilepsy have been detecting, including 1q21.1, 2q13, 9q34.3, 10q26.3, 15q11.2, 15q11-q13, 19q13.3[33–35]. The result of CGH array revealed 2q24.3 microduplication involving *SCN1A* and SCN2A from 7 patients (Figure S4). *SCN-* gene on 2q24.3 duplication has been reported in a few cases. Marini's study obsessed 2 cases related only *SCN1A* duplication at exon 8–16 and 26 with Dravet syndrome[36]. Although a duplication at multiple genes *SCN2A*, *SCN3A*, and *SCN1A* was early identified in patients with early onset developmental and epileptic encephalopathy from previous studies[37,38], duplication at 2q24.3 solely involve *SCN2A* remain less explored. The latest case report found that a girl child with DEE and intractable seizure. Consistently, this study showed two patients identified *SCN2A* duplication with EIDEE and IESS associated with resistant/less response with multiple ASM therapy. However, after introducing a ketogenic diet, the patient with ASM resistance slightly reduce seizure frequency.

A KD, characterized by a low carbohydrate content and high fat content, is a primary treatment option for epilepsy that is resistant to conventional ASMs. Numerous retrospective and prospective studies substantiate its effectiveness, revealing that approximately 30% of young patients achieve complete seizure-free survival. In comparison, approximately 60% of patients experienced significant improvement, marked by a reduced seizure frequency of more than 50%[39]. Nonetheless, the application of KDs is limited owing to challenges associated with their execution, adherence to strict dietary regimens, and occurrence of adverse side effects[40]. The exact mechanisms through which the KD protects against epilepsy are still not fully understood, and there is a lack of clarity regarding the genetic factors that influence a patient's responsiveness to the diet. With the development of modern genetic sequencing, KD has been considered the gold standard and should be prescribed early in the course of epilepsy-controlled treatment for several specific conditions, such as glucose transporter protein 1 deficiency syndrome (Glut1DS), pyruvate dehydrogenase deficiency (PDHD), EIDEE, *CDKL5* encephalopathy, Lennox-Gastaut syndrome, Dravet syndrome, and EIDEE[40]. This finding is consistent with our study, which revealed that most patients exhibited effective responses against seizures after receiving the combination of a KD and ASMs. However, two patients, #5 and #9, were classified as having Dravet syndrome and became worse

and more resistant to KD supplements combined with ASMs. Here, we present three patients, #8, #20, and #24, who underwent ACTH therapy but continued convulsions, which is consistent with the findings of previous studies[41]. Later, seizures did not appear after the patients were treated with KD or multiple ASM therapies (as summarized in Fig. 3).

Clear clinical and laboratory distinctions between DEE and ME patients facilitate their diagnosis and management. While myoclonic seizures typically suggest metabolic disorders, DEEs exhibit a wider variety of seizure types and primarily affect the nervous system[42,43]. ME often involves multiple organ systems and is indicated by distinctive laboratory findings, such as elevated lactate or ammonia levels[42]. Despite the precision of genetic analysis in guiding management, this approach has limitations, as evidenced by inconclusive results in some cases within the ME group. Nevertheless, when genetic testing identifies causative genes, as observed in three autosomal recessive cases, it enables precise genetic counseling. In DEEs, focused pharmacological treatment based on genetic understanding is vital for alleviating chronic symptoms and improving quality of life[44]. This personalized medical strategy, informed by genetic diagnostics, is increasingly critical in optimizing therapeutic efficacy and enhancing patient outcomes.

Method

Sample selection and recruitment

In 2022, a cohort of 172 patients with critical seizures, abnormal EEG and MRI findings, were recruited from the pediatric NICU at the City Children's Hospital for a prospective cohort study. These patients were initially subjected to standard first-line treatments aimed at seizure control. Clinical diagnosis was typically performed through electroencephalography (EEG) and magnetic resonance imaging (MRI). To determine the underlying etiology of seizures caused by encephalitis, polymerase chain reaction (PCR) and autoimmune antibody testing were used to assess infectious and autoimmune encephalitis, respectively. Those who expressed negative results with these tests underwent ES. The primary focus of our study was to conduct ES on patients identified within the DEE and metabolic categories. The inclusion criterion was patients who had negative results for infectious and autoimmune encephalitis and were included in the ES study. Additionally, patients must have experienced developmental setbacks or a slowdown in progress coinciding with the start of epilepsy. Individuals should also display signs that might point to a metabolic issue, including intermittent episodes of worsening health, unusually large organs, or a distinctive smell. Moreover, laboratory tests should yield unusual results, such as atypical levels of amino acids or organic acids, or unusual enzyme activity that suggests a metabolic disorder. Exclusion criteria: Patients who have epilepsy due to incidents such as a head injury, brain deterioration, infections, or immune system disorders were not included in the study.

Exome sequencing and genetic analysis

Genetic variants were discovered through the ES to examine the genetic content of genomic DNA. Peripheral blood was collected in EDTA tubes, and DNA was extracted using the QIAamp DNA Blood Kit (QIAGEN, Singapore). The genomic DNA extract was followed by DNA purification using PureLink™ Genomic DNA (Invitrogen™, USA). The DNA concentration, measured using DropSense96, ranged from 80 to 200 ng/μL, with OD values (260/280) between 1.8 and 2.0 and by the Qubit 2.0 Fluorometer. The DNA samples were then fragmented, hybridized, and captured using the Illumina Exome panel. The libraries were assessed for enrichment by qPCR, and the size distribution and concentration were determined using the Agilent 2100 Bioanalyzer. Libraries prepared for sequencing were run on a NovaSeq 6000 Sequencing System by Macrogen (Republic of Korea) using 2 × 100 bp paired-end sequencing. Quality control of the raw sequence data, including Phred-score, GC content, read length, and sequence duplication levels, was conducted using FASTQC. The Trimmomatic tool was used for trimming and quality filtering of low-quality bases and adaptor sequences. The read pairs were subsequently aligned to the human reference genome (GRCh37/hg19) using BWA-MEM, followed by additional processing with MarkDuplicates and base quality score recalibration. Variant calling of SNPs and indels in VCF files were performed using the GATK Haplotype and GATK Variant Filtration filtered the data. ANNOVAR facilitated the identification of variants utilizing databases such as dbSNP, the Genome Aggregation Database (gnomAD), UCSC RefSeq, 1000G, and ESP6500. For bioinformatics analysis and variant interpretation, we followed our previous pipeline[45], updated the ACMG criteria[46] and gene panel[47] to screen the disease–causing gene, including gnomAD v4.1.0 allele frequency and counts, ClinVAR reports, and VarSome 12.1.0 version[48]. The 3D structure of the protein was predicted using SWISS-MODEL[49], and then the mutation points were modified in The PyMOL Molecular Graphics System, Version 3.0, Schrödinger, LLC. The rarity and pathogenicity of a variant were inferred from its frequency in the population, with rarer variants considered more pathogenic. Protein damage was predicted using in silico scores from tools such as DDG, SIFT, REVEL, CADD, Polyphen2, and MetaDome[50].

Array CGH analysis

We utilized array CGH with the SurePrint G3 Human CGH Microarray Kit in 1 × 244 K (AMADID Number: 014693) (Agilent Technologies, Santa Clara, CA, USA). The test samples were labeled with cyanine 3-deoxyuridine triphosphate (Cy3-dUTP) via the SureTag DNA Labeling Kit (Agilent Technologies), while sex-matched reference DNA samples were tagged with Cy5-dUTP. Post-labeling, the DNA was purified and then mixed with Cot-1 DNA, a 10× array CGH blocking agent, and 2× HI-RPM hybridization buffer (Agilent Technologies). This mixture was then dispensed onto a microarray slide. Hybridization was conducted in an Agilent hybridization chamber set at 67 °C and 20 rpm for 24 h, followed by stringent washing with Agilent's wash buffer 1 and wash buffer 2. Finally, microarray slide images were captured using the Agilent SureScan Microarray Scanner G2505C, enabling precise analysis of genome defects.

CNV analysis was conducted using Agilent Cytogenomics v5.2.0.2 and the human genome build hg18. CNVs were categorized as either gains or losses if the region contained at least three consecutive probes with a mean log2 ratio of ±0.25. CNVs smaller than 300 kb were excluded from further analysis according to the guidelines for detecting pathogenic variants. To decode and validate the clinical significance of the identified CNVs, we tapped into a rich repository of public databases, including UCSC (http://genome.ucsc.edu, accessed in November 2023), OMIM (http://www.omim.org/), DECIPHER (http://decipher.sanger.ac.uk/), and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). Our quest was to pinpoint pathogenic CNVs, both common and rare, syndromic and non-syndromic, by cross-referencing with previously documented pathogenic CNVs pertinent to DD/CM. Novel variants of uncertain significance (VUSs) were identified by exploring genomic alterations, including microdeletions and duplications. CNVs were classified as VUSs if the genes in the region had known functions but could not be directly associated with the disease under investigation.

Quantitative PCR

To validate the duplications of CNVs detected by array-CGH, we employed quantitative real-time PCR (qPCR). Primer sets were meticulously crafted for the selected genomic regions of target genes such as *SCN3A*, *SCN2A*, *SCN1A*, *SCN9A*, and the endogenous GAPDH gene as an internal control, utilizing Primer-3 Software (V.0.4.0). Each reaction was conducted in a 10 µL final volume, containing 5 µL of SYBR-Green qPCR master mix (DNA Medical Technology, HCMC, Vietnam), 10 pmol of each primer, and 20 ng of genomic DNA. The PCR reactions were performed in triplicate on a 96-well plate using the same SYBR-Green qPCR master mix. Data collection was executed with StepOne Plus™ Real-Time PCR Systems, and the raw data was processed using Data Assist software. Analysis of the qPCR results was carried out using the ΔΔCT method, with the final data visualization achieved through GraphPad PRISM software.

Assessment of treatment outcomes

The assessment of patient progress hinges on the frequency of seizures over three months. A 'complete response' was categorized as an absence of seizures or a minimal occurrence (≤2). A 'partial response' was noted when seizures significantly reduced, yet the count remained ≤10. A 'suboptimal response' was defined as a moderate reduction, with ≤20 seizure occurrences. In cases where seizure frequency remains unchanged, the condition is termed 'resistant.' Following the commencement of treatment, physicians meticulously document the patient's progress and treatment efficacy through a standardized process, with a particular focus on the frequency of seizures. In cases where the primary treatment falls short of a complete response, the patient is subject to a comprehensive prediet evaluation by clinicians and nutritionists. This assessment encompasses a nutritional review, recording baseline weight and height, analysis of blood biochemical components such as serum lipids and albumin, and a urologic ultrasound to exclude any contraindications to initiating KD therapy. Based on these findings, we may advocate for incorporating a supplementary KD, in line with the established protocol[9], which should be administered over an additional three-month period. During this extended phase, the medical team diligently observes the patient's health, continually classifying the therapeutic outcomes using the predefined response categories.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions

YTMN and B-QV drafted the article; acquired, analyzed and interpreted the data; DKN, N-VQ and N-TH contributed to the clinical and neurological ICU; acquired the data; JH and LTB critically revised the manuscript for important intellectual content and approved the submitted manuscript; C-BB conceived the project and design; acquired, analyzed and interpreted the data; and critically revised the manuscript for important intellectual content. All the authors approved the submitted manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

All methods involved in human research participants were performed in accordance with the guidelines andregulations of the Declaration of Helsinki. Informed consent was obtained from all subjects and their legalguardians. The study received Institutional Review Board (IRB) approval number #BVNDTP–2021-09-02 by City Children's Hospital Ethics Committee. Researchers and healthcare professionals must take appropriatemeasures to protect the confidentiality of personal and medical information. The data should be anonymized ordeidentified, and no images can be obtained upon further request.

Additional information

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