The expanding field of genetic developmental and epileptic encephalopathies: current understanding and future perspectives.

Nicola Specchio[1], MD, Marina Trivisano[1], MD, Eleonora Aronica[2,3], MD, Simona Balestrini[4,5,6], MD, Alexis Arzimanoglou[7], MD, Gaia Colasante[8], PhD, J Helen Cross[9], MD, Sergiusz Jozwiak[10], MD, Jo M Wilmshurst[11], MD, Federico Vigevano[12], MD, Stéphane Auvin[13], MD, Rima Nabbout[14], MD, Paolo Curatolo[15], MD

[1] Neurology Epilepsy and Movement Disorders Unit, Bambino Gesu' Children's Hospital, IRCCS, Full Member of European Reference Network on Rare and Complex Epilepsies, EpicARE, Rome, Italy

[2] Amsterdam UMC, University of Amsterdam, Amsterdam Neuroscience, Department of (Neuro)Pathology, Amsterdam, The Netherlands

[3] Stichting Epilepsie Instellingen Nederland (SEIN), Heemstede, The Netherlands

[4] Neuroscience Department, Meyer Children's Hospital IRCCS, Full Member of European Reference Network on Rare and Complex Epilepsies, EpiCARE, Florence, Italy

[5] University of Florence, Florence, Italy

[6] Department of Clinical and Experimental Epilepsy, UCL Queen Square Institute of Neurology, London, UK

[7] Paediatric Epilepsy and Neurophysiology Dpt, Hospital San Juan de Dios, Coordinating member of the European Reference Network on Rare and Complex Epilepsies, EpiCARE, Barcelona, Spain

[8] Stem Cell and Neurogenesis Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy

[9] UCL NIHR BRC Great Ormond Street Institute of Child Health, & Great Ormond Street Hospital for Children, London, UK

[10] Research Department, The Children's Memorial Health Institute, EpicARE Member, Warsaw, Poland

[11] Department of Paediatric Neurology, Red Cross War Memorial Children's Hospital, Neuroscience Institute, University of Cape Town, South Africa

[12] Pediatric Neurorehabilitation Department, IRCCS San Raffaele, Rome, Italy

[13] APHP. Service de Neurologie Pédiatrique, Centre de référence Epilepsies Rares, Membre EpiCARE, Hôpital Universitaire Robert-Debré, Paris, France. Université Paris-Cité, INSERM Neuro Diderot, Paris, France. Institut Universitaire de France (IUF), Paris, France

[14] Reference Centre for Rare Epilepsies, Department of Pediatric Neurology, Necker–Enfants Malades Hospital, APHP, Member of European Reference Network EpiCARE, Institut Imagine, INSERM, UMR 1163, Université de Paris cité, Paris, France.

[15] Child Neurology and Psychiatry Unit, Systems Medicine Department, Tor Vergata University, Rome, Italy.

Full Professors: Eleonora Aronica, Alexis Arzimanoglou, J helen Cross, Jo M Wilmshurst, Stephane Auvin, Rima Nabbout, Paolo Curatolo.

Corresponding author:

Nicola Specchio, MD, PhD, FRCP

Neurology, Epilepsy and Movement Disorders Unit

Full Member of European Reference Network EpiCARE

Bambino Gesù Children's Hospital, IRCCS

Piazza Sant'Onofrio, 4, 00165, Rome, Italy.

E-mail: nicola.specchio@opbg.net

Abstract

Recent advancements in genetic testing technologies have revolutionized the identification of genetic abnormalities in early onset developmental and epileptic encephalopathies (DEEs). This paper provides an update on the expanding landscape of genetic factors contributing to DEEs, encompassing over 800 reported genes in recent years. The focus lies on elucidating cellular and molecular mechanisms driving epileptogenesis, with an emphasis on emerging therapeutic strategies and effective treatment options. Noteworthy novel genes linked to DEE phenotypes, such as *BRAT-1*, *GNAO1*, and gene-families such as *GRIN* and *HCN*, are explored. Understanding the network-level effects of gene variants is highlighted, paving the way for potential gene therapy applications. Given the diverse comorbidities associated with DEEs, a multidisciplinary team approach is deemed essential. Despite ongoing efforts and improved genetic testing, DEEs lack a cure, and treatment complexities persist. The paper underscores the necessity for larger international prospective studies, not only focusing on seizure outcomes but also tracking developmental trajectories.

Keywords:

Developmental and epileptic encephalopathies, Dravet syndrome, Tuberous Sclerosis Complex, targeted therapies, Autism spectrum disorder

Key messages:

- Genetic testing advancements have revolutionized the identification of abnormalities in developmental and early-onset epilepsies (DEEs).
- Over 800 genes have been reported to contribute to DEEs, shedding light on cellular and molecular epileptogenesis mechanisms.
- Novel genes like AP3B2, BRAT-1, CSNK2B, GNAO1, and gene families such as GRIN and HCN are implicated in DEE phenotypes.
- There is still need to keep disentangling phenotype-genotype correlations even after a causative genetic variant is detected.
- The high drug resistance in DEE patients has driven researchers to explore novel mechanisms of action, including targeting oxidative stress and neuroinflammation or using new therapeutic tools as antisense oligonucleotides (ASO)
- Understanding the network-level effects of gene variants opens avenues for potential gene therapy applications.
- The establishment of a multidisciplinary team approach is crucial to provide a better care to children and adolescents due to the diverse comorbidities associated with DEEs.

- Despite progress, DEEs lack a cure, highlighting the need for larger international prospective studies focusing on seizure outcomes and developmental trajectories.

Introduction

Epilepsy affects over 50 million people worldwide[1]. Developmental and epileptic encephalopathies (DEEs) are a group of severe neurodevelopmental disorders characterized by early-onset, intractable seizures and intellectual disability (ID) of variable degree, or developmental slowing, plateauing or regression, and are a major contributor to the global epilepsy burden[2]. These conditions are marked by frequent and severe epileptic activity, which not only contributes to the clinical epilepsy but also exacerbates developmental impairments. The causes of DEE are heterogeneous, but most commonly genetic[3]. These conditions most commonly result from single gene pathogenic variants that interfere with normal brain development and function, however in some cases the underlying molecular defect is not identified[4]. DEEs often manifest within the first year of life and have a profound long-term impact on the affected individuals and their families. In a recent epidemiological study the cumulative incidence for DEE has been reported as 169 of 100,000 children[5]. The clinical presentation of DEEs is highly variable among affected individuals; epilepsy is a constant feature but neurodevelopmental disorders, including cognitive, behavioral symptoms, and autism, can range from mild to severe. Epilepsy and neurodevelopmental disorders are both consequences of the underlying gene variant and or epigenetic factors involving many cellular and functional abnormalities interacting throughout the patient's life span. Early diagnosis and treatment are essential to aid reduction of the severity of symptoms and to enable the best possible outcome, even if efforts are still needed in establishing phenotype-genotype correlations[4,6].

Recently, there has been an attempt to create an etiologically driven classification of DEEs[6]. Gene variants associated with DEEs can affect various genes involved in critical processes of brain development including neuronal differentiation, synaptic function, ion channel regulation, and neurotransmitter signaling[4]. As a result, there is a wide spectrum of DEE subtypes, each associated with specific genes and distinct clinical features. Some well-known genes linked to DEEs include *AP3B2, ATP1A3, BRAT 1, CDKL5, CSNK2B, GABAAr, GNAO1, GRIN1, GRIN2A, GRIN2B, GRIN2D, HCN(1-4), KCNB1, KCNH5, KCNQ2, KCNT1, NBEA, PCDH19, SCN1A, SCN2A, SCN8A, STXBP1*[7]. Better understanding of the gene function and biological basis of the well-known monogenic DEEs may improve the overall management and prognostic information.

The clinical and economic burden in DEEs is complex and there are several implications to be considered. The first encompasses a wide range of symptoms and comorbidities, including drug-resistant seizures, cognitive and motor deficits, behavioral disturbances, and a high risk of premature mortality. The economic burden is substantial due to the lifelong need for medical and therapeutic interventions, including antiseizure medications, other specific treatments, and frequent hospitalizations. Moreover, the

costs extend beyond healthcare expenditures to include caregiving, educational support, and lost productivity for the affected individuals and their families. The economic impact is further compounded by the challenges in obtaining a timely and accurate diagnosis and the high cost of genetic testing, other investigations, and counseling[8].

The aim of this paper is to update the expanding landscape of genetic early onset DEE, highlighting the progress of knowledge in the cellular and molecular mechanisms underlying the epileptogenesis and providing a brief review of the most effective treatment options and emerging therapeutic approaches for different types of DEEs.

Search strategy

This article is based on peer-reviewed publications from January 2017 to March 2024. Search of PUBMED for the term “genetic developmental and epileptic encephalopathy” returned 2614 possible articles (accessed 31 March 2024). Our more refined search terms were “genetic developmental and epileptic encephalopathy” AND (as individual combinatory terms) “treatment”, “gene therapy”, “phenotype”, “targeted therapies”, “genes”, “gene function”, “loss of function”, “gain of function”, “diagnostic criteria”, “mechanisms”, and “neurobiology”. Selection criteria from full-text outputs were the novelty of study findings and their relevance to neurologists, with inclusion decided collectively by all authors. For clarity, relevant historical references outside the search timeframe were also included.

Epileptogenesis in the developing brain

Epileptogenesis in DEE is a complex and dynamic process involving a wide range of both gene dependent and gene-independent mechanisms that culminate in the establishment of a pathological "network" capable of generating seizures and a spectrum of associated neurodevelopmental disorders[9]. Combinatorial treatment strategies targeting various components of the epileptogenic network have been hypothesized and referred to as “Network therapy”[10].

Epileptogenesis in the developing brain is uniquely due to accelerated maturation of excitatory circuits, with transient hyperexcitability during infancy related to developmental changes of both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) and N-methyl-D-aspartate (NMDA) subunits receptors, under-developed inhibitory network with depolarizing Gamma-aminobutyric acid (GABA) currents, and the potential for long-term structural and functional modifications in the maturing nervous system[11,12].

The primary step in unraveling the mechanisms of epileptogenesis involves gaining insights into gene expression and function, identifying specific zones where they are expressed in the brain, and pinpointing the specific cell types, including excitatory and inhibitory neurons, as well as glial cells[13]. This is achieved using clinically relevant models. Activation of the mammalian target of rapamycin (mTOR) signaling in the immature developing cortex represents a shared pathogenic mechanism in a group of developmental malformations characterized by common histopathological and clinical features[14].

Molecular strategies for the diagnosis of DEE

Establishing an effective diagnostic algorithm for DEEs is imperative to ensure timely and accurate identification, facilitating prompt intervention and support. The algorithm begins with a thorough clinical evaluation, including a detailed medical history and a comprehensive neurological examination. Early recognition of seizure semiology, developmental delays, and associated comorbidities is critical. Subsequently, neuroimaging techniques, such as magnetic resonance imaging (MRI), may be employed to identify structural abnormalities in the brain[15].

Molecular strategies for the diagnosis of genetic DEEs have seen relevant advancements in recent years, revolutionizing the precision and accuracy of diagnosis (Fig.1). The identification of specific genetic variants responsible for DEEs has not only improved diagnostic accuracy but also provided valuable insights into disease mechanisms[16]. Moreover, the presence of a diagnosis of DEE substantially increases the diagnostic yield as clearly reported in a recent comprehensive systematic evidence review on 154 studies on genetic testing[17]. Rapid genome sequencing demonstrated also a high diagnostic yield and clinical utility[18].

Whole exome sequencing (WES) and whole genome sequencing (WGS) are powerful tools for identifying causal genetic variants in DEEs. WES is particularly useful for detecting variants in protein-coding regions, while WGS provides a more comprehensive view of the entire genome[19,20]. Targeted gene panels focusing on known DEE-associated genes offer a cost-effective and efficient approach to diagnosis. These panels include a selection of genes known to be implicated in DEEs, increasing the likelihood of identifying causal variants[21]. Sometimes genetic testing may reveal uncertain results, such as Variants of Uncertain Significance (VUS) which are absent from population databases and not previously reported in patients, and for which clinical role is unclear. In such cases, segregation analysis and deep phenotyping, may support the interpretation of molecular findings[22]. Functional assays, such as in vitro expression studies, can help confirm the pathogenicity of identified variants and provide insights into the molecular mechanisms underlying DEEs. Advanced

bioinformatics tools and algorithms are crucial for analyzing the vast amount of sequencing data generated in molecular diagnostics[22]. These tools help identify potentially pathogenic variants and prioritize them for further investigation.

Advancements in multiomic technologies, including transcriptomics, have substantially enhanced the genetic diagnosis of DEE by providing deeper insights into gene expression patterns and regulatory mechanisms beyond what genomics alone can reveal[23]. These integrative approaches enable a more comprehensive understanding of the molecular underpinnings of these disorders, leading to improved diagnostic accuracy and personalized therapeutic strategies[24].

In the genetic diagnosis of DEE, understanding non-monogenic contributions, such as polygenic risk scores and susceptibility factors, is crucial for identifying complex genetic interactions and multifactorial influences that contribute to disease risk and variability in clinical presentation[25,26]

Genetic diagnoses considerably influence clinical management decisions in epilepsy, with nearly half of patients experiencing changes such as medication adjustments and specialist referrals following testing[27].

The expanding field of genetic DEE

In the last few years more than 800 genes related to DEEs have been reported[28], and data should be evaluated with phenotype-genotype correlations based on detailed clinical examination, reference to validated EEG patterns whenever available, and natural history studies. In the context of DEEs, the concept of variants with a Gain-of-Function (GoF) and Loss-of-Function (LoF) impact is critical in understanding the underlying mechanisms of the genetic etiology. For example, GoF variants in ion channel genes might be managed with inhibitors that reduce activity, whereas LoF variants could be approached with strategies to boost activity or compensate for the LoF. By determining whether a variant leads to GoF or LoF, clinicians can adopt a personalized approach to treatment, which can improve outcomes and minimize adverse effects. Additionally, knowing the functional impact of a variant informs the development of model systems, such as cell lines and animal models, that simulate the disease phenotype, thereby aiding research and drug discovery. The coexistence of GOF and LOF pathogenic variants within DEEs highlights their genetic heterogeneity, suggesting that personalised medicine must consider the functional impact of variants, not just the affected gene [7].

Somatic mosaicism, involving post-zygotic mutations that occur in a subset of cells, plays a critical role in the genetic diagnosis DEE[29]. This phenomenon can result in important diagnostic challenges due to the presence of different genetic profiles within

the same individual, leading to variations in clinical severity, disease progression, and response to treatment among affected individuals[30]. Recognizing and accurately detecting somatic mosaicism is essential for developing precise diagnostic and therapeutic strategies for these complex disorders[31].

The following selected examples of genes were selected to illustrate several important themes: the complexity of pathophysiological mechanisms including functional consequences of a given variant (*SCN2A* and *SCN8A*), the challenges in identifying pathogenic somatic variants (*DEPDC5*), the necessity for natural history studies (*SCN1A*), the clinical relevance of symptoms beyond seizures (*CDKL5*), and novel treatment approaches (*KCNT1* and *SCN1A*). The diverse genetic and etiologic landscape of various epilepsy syndromes necessitates tailored diagnostic investigations, enabling precise identification of underlying genetic mutations and informing personalized therapeutic strategies[32].

Table 1 summarizes genetic and clinical findings of the most frequent DEEs, and Figure 2 shows the genetic landscape of the DEEs associated genes.

The complexity of pathophysiological mechanisms including functional consequences of a given variant

The phenotype in *SCN2A* and *SCN8A* related disorders may vary according to the functional impact of the genetic variant on the channel activity[33,34]. *SCN2A* variants with a GoF impact are associated with an early onset epilepsy, before the third month of age, often neonatal. Seizures are mainly focal with a good response to sodium-channel blockers, such as phenytoin, carbamazepine and lacosamide. In contrast, *SCN2A* pathogenic variants with a LoF effect are associated with a later onset of epilepsy, mainly characterized by generalized tonic-clonic seizures and myoclonic seizures, and poor response to sodium-channel blocker drugs[35]. In *SCN8A*-related disorders Self-limited infantile epilepsies and DEE are both caused by pathogenic variants with a GoF effect, while a later onset of generalized epilepsy and neurodevelopmental disorders without epilepsy are caused by LoF variants[33,34].

The challenges in identifying pathogenic somatic variants

Germline LoF *DEPDC5* variants cause various epilepsy phenotypes transmitted in an autosomal dominant manner with incomplete penetrance[36]. Germline *DEPDC5* variants have been identified in patients with focal cortical dysplasia, particularly type II (FCD II)[36,37]. DEPDC5 is a negative regulator of the MTOR pathway, and somatic loss of heterozygosity is likely required to develop FCD via a second hit mechanism[38]. However, relevant challenges exist in detecting somatic variants from brain samples. This difficulty may stem from the presence of the second hit in an intron or a different gene affecting the same pathway. Additionally, detecting somatic second hit variants can be challenging if the sampled tissue exhibits low mosaicism. In the future, this challenge

can be addressed using specialized molecular methods (e.g. ultra-deep sequencing or single-cell sequencing)[39].

The necessity for natural history studies

The establishment of patient registries is paramount for advancing the understanding and management of DEEs. As an example, in patients with *SCN1A* genetic variants, has been developed a registry, "The Italian Registry of Dravet Syndrome and Other Syndromes correlated with SCN1A and PCDH19 mutation" known as RESIDRAS[40]. RESIDRAS systematically collects extensive patient data, encompassing genetic profiles, detailed clinical phenotypes, treatment regimens, and long-term outcomes, thus creating a robust resource for both research and clinical practice. RESIDRAS provides valuable insights into the natural history of Dravet syndrome, revealing critical periods for therapeutic intervention. For instance, early initiation of preventive treatments has been shown to mitigate cognitive decline and reduce seizure severity. Additionally, the registry data enable large-scale epidemiological studies, contributing to the identification of potential biomarkers for disease progression and therapeutic response. RESIDRAS provided information on mortality rate which was of 1.84 (95% CI 0.77–4.42) per 1000-person-years[41]. By centralizing patient information, RESIDRAS not only enhances the precision of clinical care but also accelerates research efforts.

The clinical relevance of symptoms beyond seizures

LoF variants in *CDKL5* are repsosible of CDKL5 deficiency disorder (CDD)[42]. While seizures are a hallmark of CDD, patients often experience a broad spectrum of symptoms that drastically impact their quality of life. These include severe cognitive and motor impairments, autistic traits, gastrointestinal issues, sleep disturbances, and visual dysfunction[43]. Although CDD is not considered a neurodegenerative disorder, emerging evidence based on quantitative MRI analysis suggest a progressive brain atrophy[44].

Novel treatment approaches

KCNT1 pathogenic variants are generally missense and lead to disease through GoF mechanisms including increases in current magnitude and or current–voltage relationships. Targeted treatment with quinidine, a class I antiarrhythmic drug, has been used to block hyperexcitable *KCNT1* channels, but with variable results[45]. Its variability of efficacy can be partly explained by dose-limiting off-target effects, poor blood-brain barrier (BBB) penetration, and low potency[45].

Antisense oligonucleotide (ASO) therapy represents a promising treatment approach for Dravet syndrome, particularly targeting the underlying genetic variant in *SCN1A*. The antisense oligonucleotide STK-001, also known as ASO-22, employs targeted augmentation of nuclear gene output technology to prevent the inclusion of the exon

20N, which triggers nonsense-mediated decay, in the human SCN1A gene. This approach effectively enhances the production of functional Scn1a transcripts and Nav1.1 channel expression. In preclinical studies using a mouse model of Dravet syndrome, STK-001 treatment decreased the occurrence of electrographic seizures and sudden unexpected death in epilepsy[46]. Data from the phase 1 and 2a MONARCH clinical trial (NCT04442295) and ADMIRAL clinical trial (NCT04740476) revelead positive preliminary results on seizures reduction.

The concept of Prediction and Prevention

Prediction models in DEEs have made substantial strides in recent years, providing valuable insights into disease prognosis and personalized care strategies. These models can estimate the likelihood of a specific genetic variants contributing to a DEE based on the patient's clinical presentation, family history, and specific EEG patterns[47]. Notably the application of deep learning techniques has shown promise in identifying subtle EEG patterns that may not be evident for human observers[48].

Considering a patient's genetic variants, previous treatment responses, and comorbidities, can assist in selecting appropriate anti-seizure medications (ASMs) or other therapeutic interventions[49]. Furthermore, we can estimate the likelihood of a patient experiencing specific side effects or complications based on their unique genetic profile, allowing for more precise and individualized care[49]. The ongoing development and refinement of DEE prediction models hold the potential to remarkably enhance clinical decision-making and prediction of patient outcomes.

Integrating electro-clinical and genetic data through phenotype-genotype correlation is essential for interpreting genetic findings in the context of a specific clinical presentation[22].

A prediction model recently established the estimation at disease onset of patients with *SCN1A*-related epilepsies enabling the distinction between Dravet Syndrome (DS) vs Genetic Epilepsy with Febrile Seizure *plus*, assisting clinicians with prognostic counseling and supporting the early institution of precision therapies (http://scn1a-predictionmodel.broadinstitute.org/)[50].

The concept of prevention of epileptogenesis broadens the scope of therapeutic interventions. Beyond the goal of preventing the onset of epilepsy, the concept of "disease (or syndrome) modification" in epilepsy extends to altering antiepileptogenesis, modifying comorbidities, or addressing both aspects[51–53]. To prevent epileptogenesis and modify the disease natural history, it is essential to understand the various intrinsic and extrinsic factors involved in the different steps of the epileptogenic process, as well as the development of comorbidities and drug

resistance. This is particularly challenging in genetic epilepsies, such as Tuberous Sclerosis Complex (TSC), in which we are often confronted with an 'immediate and early" or even "prenatal" epileptogenesis[53]. Data from the EpiSTOP and PREVeNT studies suggested that it is possible to delay the epilepsy onset and mitigate the overall severity of epilepsy with preventative treatment with vigabatrin administered in early infancy before the onset of seizures; however, the cognitive and behavioral impairment may not improve with such preventative strategy[54–56]. The age at onset of preventive intervention seems to be one of the most important factors in the prevention of epilepsy[56,57].

The initiation of intensive environmental enrichment and early parent mediated behavioral intervention before the age of 24 months is of crucial importance to stimulate brain plasticity, in social brain circuitry, and to foster more typical communication and developmental patterns[58].

Also in the concept of prevention we should consider Sudden Unexpected Death in Epilepsy (SUDEP) represents a relevant and often underrecognized risk in individuals with various genetic developmental and epileptic encephalopathies[59]. Research has shown that certain genetic mutations, particularly those affecting ion channels, neurotransmitter receptors, and other critical neuronal functions, can predispose patients to a higher risk of SUDEP[60]. Basic science research, including studies using animal models like mice with specific gene knockouts and advanced cellular models such as induced pluripotent stem cells (iPSCs) derived from patients, has been pivotal in revealing the pathophysiological mechanisms behind SUDEP[61].

New and emerging mechanisms-based pharmacological treatment options.

Table 2 provides a summary of the mechanism of action and the level of evidence of emerging treatment options for DEEs.[62–70] Future efforts should be directed at further exploring the possibility of applying a disease modifying treatment to patients with DEEs, and gene therapy may be a promising opportunity for such a goal.

ASO treatment approach

A post-transcriptional strategy aiming at promoting an increase of the productive splicing of Scn1a by an antisense oligonucleotide (ASO) preventing the incorporation in the mature transcript of a "poison exon", has been proved to ameliorate DS symptoms, particularly when delivered in perinatal pups[71]. Positive results from preclinical studies, combined with the relatively low invasiveness of delivering ASOs via intrathecal injection, have driven the development of the first clinical study for DS patients. This Phase 1-2b study has now recruited and treated several patients across the US and Europe.

ASO based therapies have been proven to be effective also in DEEs determined by GoF variants, such as *SCN8A* encephalopathy. ASO mediated reduction of Scn8a transcript in this model resulted in delayed onset of seizures and extended survival[72]. Interestingly, it also improved DS mice phenotype, likely because it can reduce excitatory neuron activity and rebalance altered neuronal circuitry consequent to GABAergic interneuron dysfunction[72].

Gene therapy: where we are?

Despite recent approval of new pharmacological treatments that have greatly improved the quality of life for some patients with genetic DEEs, a substantial proportion remain unresponsive to existing treatments, requiring alternative therapies. Research activities are shifting towards the direct correction of the underlying genetic cause for monogenic DEEs. Different gene therapy approaches are under development at a preclinical level in DEEs and for some clinical trials are already ongoing[73].

Gene supplementation mediated by Adeno Associated Vectors (AAVs)

Thanks to substantial advancements in the development of effective viral vectors, gene therapy in the central nervous system (CNS) has emerged as a promising approach. Adeno-associated viral vectors (AAVs) are increasingly used as delivery tools, due to their excellent clinical safety record, minimal pathogenicity, and ability to maintain stable, non-integrating expression in vivo. Efforts to enhance transduction efficiency and specificity led to the development of AAV variants capable of crossing the BBB in specific mouse strains after intravenous administration. These variants were able to broadly transduce both neurons and astrocytes throughout the CNS[74,75], which has been widely exploited in various preclinical studies to demonstrate the feasibility of gene therapies for different neurological disorders. Given that systemic administration into the bloodstream is an appealing solution as gene therapy vectors could be non-invasively delivered throughout the body, different laboratories and companies are actively working on new engineered AAVs able to effciently cross the BBB in primates[76]. Given the ongoing efforts, it is only a matter of time before variants suitable for clinical applications in humans are identified.

A traditional gene supplementation approach mediated by AAVs, specifically of PHP.B serotype, was exploited to deliver the human *CDKL5* in a CDD mouse model[77]. Among the different CDKL5 transcriptional isoforms, isoform 1 was selected for gene supplementation since it is the most enriched in the brain. Intrajugular injection of *CDKL5_1* in Cdkl5 knock out juvenile (postnatal day 30, P30) induced a marked improvement in motor function compared to control treated mice[77], that well correlated with high level of transduction in the cerebellum. When delivered in vitro, in neurons differentiated from *CDKL5*-deficient patient derived human-induced pluripotent stem

cells (h-iPSCs), isoform 1 was able to reinstate synaptic function in neurons while isoform 2 ameliorated the calcium signaling defect[77].

In the case of TSC, potential treatment involves the supplementation of hamartin. In mouse models with Tsc1 variants, the delivery of human *TSC1* gene through retro-orbital injection of AAV9 at P2171) was able to improve brain pathology biomarkers, enhance rotarod performance, and increase lifespan[78]. It is likely that the new AAV variants will further enhance brain cell transduction leading to improved symptomatic recovery.

Alternative viral vectors and strategies for larger coding sequences

For larger coding sequences, such as those encoding for voltage gated sodium channels (NAV, ~6 kbps), AAVs are not well-suited due to their payload limitations (4.8-5 kbps cargo capacity). Thus, for DEEs caused by variants in those genes, alternative therapeutic options are being developed.

Further advances of developments of viral vectors with higher payload have been successfully exploited for the delivery of a stabilized coding sequence (CDS) of *SCN1A* in DS mouse model at symptom onset[79] or after[80]. When *SCN1A* cds was delivered by Helper-dependent (HD) adenoviral vectors in basal ganglia, cerebellum, and prefrontal cortex of DS mice at 5 weeks of age when most neurological alterations were present, mice gained modest attenuation of the characteristic phenotype[80]. More recently, the same optimized CDS was delivered by Canine Adenovirus (CAV-2), that preferentially transduce neuronal cells and exert a robust retrograde axonal transport. Local viral injections in the thalamus and hippocampus of DS mice resulted in transduction also of the connected brain regions with consequent improvement in the survival of the mice, reduction of spontaneous seizures, increase of temperature threshold for hyperthermic-induced seizures and with some effect on behavioral deficits[79]. Some effect, although more modest, was achieved when the same treatment was delivered after symptom onset.

To overcome the challenge of CDS size, another possibility is to rescue gene haploinsufficiency by boosting the expression of the healthy allele of the gene. This strategy has been exploited to enhance Scn1a gene transcription, using activator dCas9 system (CRISPRa)[81] or zinc finger activator proteins119 targeting specifically Scn1a gene promoter and delivered in DS mice by AAVs, under the control of GABAergic specific regulative regions[81,82]. Those transcriptional strategies present the main advantage to finely tune the endogenous expression of the gene, conserving the different splicing isoforms and most of the post transcriptional regulation to which it might be subjected. They achieved relevant decrease in hyperthermic-induced seizures when delivered in the pre-symptomatic stage in DS mice[81,82]. Optimization of those

approaches is being performed and the interest of some companies will hopefully lead to the development of clinical trials.

Current challenges and future directions

Since DEEs often present with a wide spectrum of associated comorbidities, a multidisciplinary team approach is indispensable in addressing the complex needs of individuals with genetic DEEs. The team's collective expertise ensures accurate diagnosis, personalized treatment plans, and ongoing support for individuals with DEEs and their families. Furthermore, this approach facilitates the integration of clinical care with research, fostering advancements in understanding and managing DEEs.

In this section, we discuss several key challenges currently faced in the field and propose potential future directions. These observations are primarily based on the authors' professional experiences and insights. The proposed future directions provide a roadmap for advancing the field and achieving sustainable growth.

The establishment of specialized clinics dedicated to genetic DEEs could serve as a critical bridge between scientific advancements in genetics and the practical application of precision medicine. One of the paramount needs met by these clinics is the provision of accurate genetic diagnosis through advanced sequencing techniques such as WES and WGS that may confirm the presence of DEEs, consider known alerts related to phenotype-genotype correlations and, guide personalized treatment strategies. Further early recognition of comorbidities with intervention can be offered.

In addition to medical care, specialized DEE clinics should offer invaluable emotional support, counseling, and education for individuals helping families to understand the genetic basis of DEEs, recurrence risks, available testing and treatment options. DEE clinics should also serve as advocacy and networking hubs, connecting families with resources, support groups, and organizations focused on DEEs.

Diagnosing DEEs in low and middle-income countries (LMICs) can be particularly challenging due to limited resources and restricted access to advanced medical technologies. Therefore, molecular strategies for DEE diagnosis in LMICs should be both cost-effective and efficient. One approach is to implement targeted Next Generation Sequencing panels designed to identify variants in known genes associated with DEEs, focusing on prevalent variants that occur within the population. Additionally, leveraging telemedicine and online collaboration with genetic experts from higher-income regions can aid in remote interpretation of genetic data.

The establishment of centralized registries with well-delineated phenotypes and accurately diagnosed patients with epilepsy is crucial for advancing research, enhancing treatment strategies, and promoting collaboration among healthcare

professionals. EpiCARE, a European Reference Network on rare and complex epilepsies, serves as an exemplary model, exemplifying the relevance of pooling standardized data to deepen our understanding of epilepsy, refine diagnostic criteria, and ultimately improve the care and outcomes for individuals affected by this complex neurological condition.

There is presently no cure for DEEs, and treatment can be difficult with current ASMs. Clinical trials for genetic DEEs face several important challenges that can impact their design, execution, and interpretation. One of the primary challenges lies in the rarity and heterogeneity of DEEs, making it challenging to recruit enough participants for clinical trials. This rarity also means that there is limited natural history data available for many DEEs, making it difficult to determine appropriate trial endpoints and assess treatment efficacy. In this context, N-of-1 trials are particularly appropriate as they represent randomized, prospective, controlled, multiple crossover trials to test targeted treatment in a single patient with rare genetic DEEs.

Comprehensive outcome measures that extend beyond assessing seizure frequency are crucial for capturing the multifaceted impact of these complex disorders. Cognitive and developmental assessments, including standardized neuropsychological tests, adaptive behavior scales, and assessments of communication skills and motor function, provide a more holistic understanding of treatment effects.

While epilepsy management in DEE remains challenging due to drug resistance, prospective neuropsychological studies indicate that developmental abnormalities can be improved through Early Environmental Enrichment, mediated by parents. Such enrichment can enhance communication, relationships, and stimulate social brain circuits.

Quality of life measures and caregiver-reported outcomes also offer valuable insights into the broader impact of DEEs on the lives of affected individuals and their families, helping to guide therapeutic decision-making and improve the overall clinical trial design. By incorporating these diverse outcome measures, researchers aim to better evaluate the overall effectiveness of interventions and address the complex neurodevelopmental challenges that individuals with DEEs often face.

Additionally, the genetic complexity of DEEs presents challenges in identifying suitable therapeutic targets for drug development. While some DEEs are caused by well-defined genetic variants, others may result from a combination of genetic and environmental factors. Exposure to inflammation has been shown to influence the expression and function and to exacerbate the effects of *SCN1A* gene variants. This exacerbation can lead to a worsening of clinical symptoms, particularly in conditions such as Dravet syndrome potentially increasing the frequency and severity of seizures and other neurological deficits.

Characterizing the specific effects of gene variants at the network level has paved the way in determining the biological convergence in DEE phenotypes[83]. Identifying specific molecular targets for intervention can be challenging, and therapies developed for one genetic subtype may not be effective for others. Studies, such as the recently published multi-omic analysis of blood from TSC infants of EPISTOP trial will be of utmost value[84]. Overcoming these challenges will require close collaboration between researchers, clinicians, and geneticists, as well as innovative trial designs that consider the unique characteristics of DEEs.

Larger international prospective studies are needed to follow not only the seizure outcome but also the developmental trajectories. Early genetic diagnosis should be provided to all patients to enable the enrollment in such studies.

Once the underlying genetic variants is identified, precision medicine opens the door to targeted therapies as in certain DEEs associated with mTOR overactivation aimed at modulating the molecular pathways affected by the variants are being explored. This approach leverages individualized genetic information to tailor diagnostic and therapeutic strategies, offering new hope for patients and families affected by these severe neurological disorders. While challenges remain, ongoing research and clinical advancements continue to expand the role of precision medicine in improving the lives of individuals and families affected by DEEs. This approach is still in its infancy but can gradually benefit from the reorganization of clinics for DEEs according to the new development of medicine in the specific field. Collaboration of a multidisciplinary working team is essential to strength the efforts to advance research against these devastating conditions.

A number of short- and long-term goals should be considered in the future strategies to improve and optimize the management of patients with DEEs.

Short-term goals (within 1-5 years) are: (1) Investigating how genetic variants impact cellular function and structure, studying the predictive value of genetic variants on brain pathology and early neurological development; (2) building up an international registry of rare DEE collaborating with global partners to collect and maintain data; (3) develop a comprehensive genomic database and apply advanced data analysis techniques for genetic research.

Long-term goals (beyond 5 years) are: (1) implementation of gene therapy as a potential treatment option for DEE; (2) research and identify biomarkers that can predict the rate of disease progression, severity of epilepsy, and the emergence of comorbid conditions; (3) conduct international clinical trials to evaluate the efficacy of treatments targeting the biological mechanisms underlying DEE; (4) combine data from multiple omics disciplines, such as genomics, proteomics, and metabolomics, to gain a comprehensive understanding of DEE pathogenesis.

Achieving these short-term and long-term goals requires a coordinated effort among various stakeholders, each bringing their expertise and resources to the table, including clinicians, researchers, IT specialists, AI researchers, pharmaceutical industry R&D, biotech companies, policy makers, and international organizations.

Addressing the challenges of DEEs requires a strong, coordinated effort. By combining clinical care with advanced research, we can better understand and manage these conditions. Establishing specialized clinics, improving diagnostics, and fostering international collaborations are crucial steps. Putting together diverse experts will help create a sustainable framework to support individuals with DEEs and their families. Our commitment to this mission ensures that scientific progress translates into effective, personalized care for those affected by these rare and severe disorders.

Contributors

Nicola Specchio and Paolo Curatolo contributed with methodology, conceptualisation, visualisation, writing original draft, and writing review & editing.

Marina Trivisano, Eleonora Aronica, Simona Balestrini, Gaia Colasante, Sergiusz Jozwiak, Stéphane Auvin contributed with writing original draft, literature search, writing review & editing.

Alexis Arzimanoglou, J Helen Cross, Jo M Wilmshurst, Federico Vigevano, Stéphane Auvin, Rima Nabbout contributed with literature search, review & editing.

Declaration of Interest

NS has served on scientific advisory boards for GW Pharma, BioMarin, Arvelle, Marinus and Takeda; has received speaker honoraria from Eisai, Biomarin, Livanova, Sanofi; has served as an investigator for Zogenix, Marinus, Biomarin, UCB, Roche.

MT has served in advisory boards for BioMarin and Biocodex; has received speaker honoraria from BioMarin, Biocodex and Orion; has served as an investigator for Zogenix, Marinus, Biomarin, UCB, Roche.

EA has no competing interests.

SB has served in advisory boards for Biocodex; has received speaker and consultant honoraria from Angelini, Biocodex and Jazz Pharmaceutics.

AA has received speaker and consultant honoraria from Biocodex, EISAI, Jazz Pharmaceutics, Sanofi, UCB. AA is Co-director European Consortium for Epilepsy Trials (ECET).

GC has no competing interests.

JH Cross has received grants from Stoke Therapeutics, Ultragenyx, UCB, National Institute for Health and Care Research (NIHR), Great Ormond Street Hospital Children's Charity (GOSHCC), LifeARC, the Waterloo Foundation and the Action Medical Research. JHC has also received honoraria payments from Biocodex, Nutricia, Jazz Pharmaceuticals, Nutricia, Takeda and UCB, all of which have been paid to University College London.

SJ has no competing interests.

JMW is Associate Editor for Epilepsia and Chief Editor Paediatric Neurology subsection of Frontiers in Neurology. JMW has served in advisory boards for Sanofi and Novartis.

FV has received speaker fees from Zogenix, Neuraxpharm, Angelini and Eisai; has served in advisory boards for Zogenix, Neuraxpharm, Angelini and Eisai.

SA received honoraria for lectures from Biocodex, Biomarin, Eisai, Jazz Pharmaceuticals, Neuraxpharm, Nutricia, Stoke, UCB Pharma, Zogenix. He has be paid as a consultant for lectures from Biocodex, Encoded, Grintherapeutics, Jazz Pharmaceuticals, Neuraxpharm, Nutricia, Orion, Proveca, Supernus, Stoke, Takeda, UCB Pharma, Xenon. He has been an investigator for clinical trials for Eisai, Proveca, Takeda, UCB Pharma.

RN has served as principal investigators in clinical trials for Novartis, Nutricia, Eisai, UCB, GW Pharma, Livanova. She received consulting and lecturer honoraria from Biogene, BioMarin, Praxis, GW Pharma, Zogenix, Novartis, Nutricia, Stoke, Ionis, Targeon, Neuraxpharma, Takeda, Nutricia, Biocodex, Advicennes and Eisai. She received unrestricted research grants from Eisai, UCB, Livanova and GW Pharma and academic research grants from European Joint Programme on Rare Diseases (EJP-RD) (Horizons 2020).

PC has served on scientific advisory board for Novartis, has received speaker honoraria from Jazz Pharmaceuticals and ItalFarmaco, has served as investigator for clinical trials for Novartis.

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We confirm that this paper has not been submitted to another journal and has not been published in whole or in part elsewhere previously.

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Figure Legend

Figure 1. Molecular strategies for the diagnosis of genetic DEEs.

Figure 2. Circus plot (implemented using R software) illustrating the relationship between different genes (blue) and their associated epilepsy phenotypes (green). The plot visualizes the connections between gene mutations (e.g., SCN1A, SCN2A) and their respective abnormal functions, highlighting the genetic complexity of epilepsy. The levels of the variable 'Epilepsy_Phenotype' have been abbreviated. The abbreviations used are as follows: DS (Dravet Syndrome), GEFS+ (Generalized Epilepsy with Febrile Seizures Plus), EIDEE (Epileptic Encephalopathy with ID and Autism), FE (Bi-temporal Focal Epilepsy), Epi with or without ID-Autism (Epilepsy with or without Intellectual Disability and Autism Spectrum Disorder), SeLNE (Severe Late-onset Neonatal Epilepsy), SelFIE (Self-limited Focal Infantile Epilepsy), PCDH19-CE (PCDH19-Clustering Epilepsy), FS (Simple Febrile Seizures), iDEE (Infantile DEE), LKS (Landau-Kleffner syndrome), iEpi (Infantile onset epilepsy with both focal and generalized seizures), DR (Drug-responsive or self-limited focal or generalized epilepsy), DEE+dys (DEE and late onset dystonia), MD with or without Epi (Early onset hyperkinetic movement disorder associated with epilepsy). In the legend is represented the abnormal_function indicates the nature of the genetic abnormality. The colors used for the connections in the circus plot correspond to Orange for LOF (haploinsufficiency Insufficient + Loss of Function), Violet for GOF (Gain of Function), Pink for CI (Complex Inheritance), Green for GOF+LOF (Gain of Function + Loss of Function).

DEE	CODIFY	ABNORMAL PROTEIN	ABNORMAL FUNCTION	EPILEPSY PHENOTYPE	MAIN CLINICAL FEATURES	TARGETED TREATMENTS
SCN1A	Voltage-gated sodium channel, Nav1.1	Reduces action potential firing in GABAergic inhibitory interneurons	haploinsufficiency and LoF	Dravet Syndrome GEFS+	Fever sensitivity Seizure onset < 6m Ataxia Mild-severe ID ASD	FFA established use in clinical practice. Under exploration the possible effect as disease modifying treatment. Avoidance of sodium channel blockers
			GoF	EIDEE Bi-temporal focal epilepsy	Neonatal onset seizures Movement disorder Congenital Arthrogryposis Severe-profound ID	Sodium channel blockers established
SCN2A	Voltage-gated Sodium channel Nav1.2	Regulating the flow of sodium ions across neuronal membranes	haploinsufficiency and LoF	Epilepsy with or without ID and ASD	Infantile onset, mainly generalized and myoclonic seizures	Avoidance of sodium channel blockers
			GoF	SeLNE SeLFNE EIDEE	Epilepsy onset < 3m, often neonatal, with mainly focal seizures Severe-profound ID in EIDEE	Sodium channel blockers established, ASO treatment under exploration
SCN8A	Voltage-gated Sodium channel Nav 1.6	Regulating the membrane excitability of neurons	haploinsufficiency and LoF	Infantile onset epilepsy with or without ID and ASD	Infantile onset, mainly generalized and myoclonic seizures Mild-severe ID ASD	Avoidance of sodium channel blockers
			GoF	SeLFIE SeLIE EIDEE	Epilepsy onset < 3m, often neonatal, with focal and generalized seizures	Sodium channel blockers established

					Severe-profound ID in EIDEE	
PCDH19	Protocadherin19	Altered synaptic transmission due coexistence of mutated protocadherin-19 and wild-type neurons	Cellular interference	PCDH19-Clustering Epilepsy	mainly female subjects seizure clusters fever sensitivity mild-moderate ID ASD	Ganaxolone under exploration
STXBP1	Syntaxin-binding protein 1, Sec1, Munc18-1	Munc18-1 is a key organizer of the neuronal SNARE complex, the molecular machine driving synaptic vesicle fusion	haploinsufficiency and LoF	IESS EIDEE	Seizure onset < 1y (90%) Moderate-severe ID Movement disorders	Phenylbutyrate under exploration (https://classic.clinicaltrials.gov/ct2/show/NCT04937062062)
CDKL5	Cyclin dependent kinase-like 5 (CDKL5)	CDKL5 is localized at excitatory synapses and contributes to correct dendritic spine structure and synapse activity	haploinsufficiency and LoF	EIDEE	Infantile-onset refractory epilepsy Movement disorders Hypotonia Moderate-severe ID ASD Central visual impairment	n.a.
KCNQ2	Voltage-gated potassium channel, subunit Kv7.2	Regulating the membrane excitability of neurons	haploinsufficiency and LoF	EIDEE SeLFIE SeLIE	Neonatal onset epilepsy, with focal seizures Severe-profound ID	Sodium channel blockers established
GABAA-R (GABRA1-5, GABRB1-3, GABRG2, GABRD)	γ-Aminobutyric acid type A (GABAA) receptors are formed by a pentameric assembly from 19 different subunits, with	GABAA receptors represent the major class of inhibitory ion channels in the mammalian brain	GoF and LoF	Ranging from simple febrile seizures or GGE to severe DEEs, such as EIMFS, IESS, and LGS		GoF: nitrazepam, phenobarbital LoF: vigabatrin established; vinpocetine under exploration

	most receptors containing two α-subunits, two β-subunits, and a γ or δ subunit					
GRIN (GRIN1, GRIN2A, GRIN2B, GRIN2D)	N-methyl-d-aspartate (NMDA) receptors	Mediate a slow component of excitatory synaptic transmission	GoF and LoF	GRIN1: EIDEE GRIN2B: DEE, neurodevelopmental disorder with or without epilepsy, schizophrenia. GRIN2A: epileptic spectrum ranging from Landau-Kleffner syndrome to epilepsy with CT spikes and speech or language impairment, intellectual disability or developmental delay often in comorbidity GRIN2D: DEE		GoF: memantine, radiprodil under exploration LoF: D-serine established
BRAT1	Nuclear protein that interacts with the tumor suppressor protein BRCA1 (breast cancer 1) and ATM (ataxia telangiectasia mutated).	Essential for mitochondrial function, cell proliferation, and cell cycle progression by recruitment of growth factors	LoF	RMFSL EIMFS NEDCAS	Microcephaly Bradycardia Cardiac arrest leading to early death	n.a.
CSNK2B	Regulatory subunit of casein kinase II (CK2), a ubiquitous serine and threonine kinase complex	Takes part in various signalling pathways, including NF-kB, PTEN, PI3K-Akt and Wnt-b-catenin	haploinsufficiency and LoF	Infantile onset epilepsy with both focal and generalized seizures	Hypotonia Dysmorphism Mild-severe ID Behavioral disorders	n.a.
NBEA	Neurobeachin, a brain-specific kinase-anchoring protein	Implicated in vesicle trafficking and synaptic structure and function	haploinsufficiency and LoF	Early childhood-epilepsy. EMAtS-like phenotype	PKD ASD	n.a.

KCNT1	Sodium-activated potassium channel	regulates neuronal excitability by contributing to the resting membrane potential and hyperpolarisation following a train of action potentials.	GoF	EIFMS SHE DEE	EIFMS SHE (sleep related seizures, sleep and psychiatric disorders)	Fluoxetine under exploration and Quinidine established
KCNB1	α subunit of the voltage-gated potassium channel subfamily 2 (Kv2.1)	regulating excitability in pyramidal neocortical neurons	GoF	DEE	Epileptic spasms, and generalized and focal seizures Early DD (mostly severe to profound) ASD	n.a.
			LoF	DE with or without epilepsy	Less frequent and less severe epilepsy	n.a.
HCN(1-4)	Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels	Key role in the regulation of cellular excitability, synaptic integration, and neuronal network dynamics	GoF or dominant negative effects (HCN1-2), HCN3 not available, HCN4 GoF and LoF	CAE FS and GEFS+ GGE DEE	Generalized seizures ID and ASD	Ivabradine, a pan-HCN channel blocker approved for heart failure, under exploration
KCNH5	Ether-a-go-go (EAG2) voltage-gated potassium channels Kv10.2	Localizes to the somatodendritic region where it plays a role in controlling the electrical coupling between cell bodies and distal dendrites	GoF	EIDEE Drug-responsive or self-limited focal or generalized epilepsy with normal cognitive outcome	Infantile onset epilepsy, focal and generalized seizures	n.a.

GNAO1	G-protein α subunit that forms a heterotrimeric G-protein complex	Role in transmembrane signaling pathways	haploinsufficiency and LoF	DEE and late onset dystonia		n.a.
			GoF	Early onset hyperkinetic movement disorder associated with ID, with or without epilepsy		n.a.
AP3B2	Heterotetrameric adaptor protein (AP) complexes (AP1-5)	Key role in signal-mediated trafficking of integral membrane proteins	LoF	EIDEE	Optic atrophy Microcephaly Severe-to-profound DD Movement disorders	n.a.

Table 1. Summary of the main genetic and clinical findings of the most frequent DEEs
GoF = Gain of Function; LoF = Loss of Function; EIDEE = Early Infantile Developmental and Epileptic Encephalopathies; DEE = Developmental and Epileptic Encephalopathies; CAE = Childhood Absence Epilepsy; LGS = Lennox Gastaut Syndrome, EIMFS = Epilepsy of Infancy with Migrating Focal Seizures; IESS = Infantile Epileptic Spasms Syndrome; FS = Febrile Seizures; GEFS+ = Genetic Epilepsy with Febrile Seizures plus; EMAtS = Epilepsy with Myoclonic-Atonic Seizures; SHE = Sleep-related Hypermotor Epilepsy; GGE= Genetic Generalized Epilepsy; ID = Intellectual Disability; DD = Developmental Delay; ASD = Autism Spectrum Disorder; PKD = Paroxysmal Kinesigenic Dyskinesia; SeLNE = Self-limited Neonatal Epilepsy; SeLFNE = Self-limited familial neonatal epilepsy; SeLIE = Self-limited infantile epilepsy; SeLFIE = Self-limited familial infantile epilepsy; RMFSL = Lethal Neonatal Rigidity and Multifocal Seizure Syndrome; NEDCAS = Neurodevelopmental Disorder with Cerebellar Atrophy and with or without Seizures; n.a. = not available; CT =Centrotemporal; FFA = fenfluramine; ASOs = antisense oligonucleotides; m = months.

Table 2 Summary of the mechanism of action and the level of evidence of emerging treatment options

Drug	Mechanism of Action	Grade of recommendation	FDA and EMA Approval	Studies in Genetic DEEs	Minimal effective dose	Clinical Trial.gov
Ganaxolone	Positive allosteric modulator of $GABA_A$ receptors synthetic analogue of allopregnanolone	A	EMA and FDA	Marigold study (NCT03572933) phase 3	33 mg per kg per day or 900 mg per day	https://clinicaltrials.gov/study/NCT03572933
Locarserin	Selective serotonin receptor (5-HT2C) agonist	B	No	Retrospective case series of 35 patients with DS (n=20), LGS (n=9) and other treatment-resistant focal epilepsies (n=3) and generalized epilepsies (n=3) Phase III trial of adjunctive lorcaserin for the treatment of patients with DS and Other Refractory Epilepsies (MOMENTUM 1; NCT04572243 and MOMENTUM 2 NCT044576879 currently ongoing	Retrospective studies: 15 mg per day Phase III trials MOMENTUM: participants weighing 10 kg to < 20 kg, 20 kg to < 40 kg, and ≥ 40 kg received 5 mg, 10 mg, and 20 mg per day, respectively	https://clinicaltrials.gov/study/NCT04572243
Soticlestat	Selectively inhibitor of cholesterol 24-hydroxylase (CH24H), a brain-specific enzyme that converts cholesterol into 24S-hydroxycholesterol (24HC), an endogenous positive allosteric modulator of the NMDA sub-type of glutamate receptor.	B	No	Phase II trial in paediatric patients with DS and LGS (the ELEKTRA study) (NCT03650452) Phase Ib and IIa study in adults with DEEs (NCT03166215) Phase III trials of soticlestat as adjunctive therapy in children and young adults with DS (NCT04940624) and LGS (NCT04938427) Phase II study in paediatric and adult patients with 15Q Duplication Syndrome (Dup 15q) and CDD (ARCADE) (NCT03694275)	In patients weighing ≥60 kg, soticlestat was titrated up to 300 mg BID, with weight-based dosing used for those weighing <60 kg.	https://clinicaltrials.gov/study/NCT03650452 https://clinicaltrials.gov/study/NCT03166215 https://clinicaltrials.gov/study/NCT04940624 https://clinicaltrials.gov/study/NCT04938427 https://clinicaltrials.gov/study/NCT05064878
Fenfluramine Hydrochloride	Increases extracellular serotonin levels, and also acts as both a serotonergic 5-HT2 receptor agonist and σ1 receptor antagonist.	B	Yes, approval only for DS and LGS	Phase III study in paediatric and adult patients with CDD (NCT05064878), active, recruiting.	Fenfluramine hydrochloride 0.8 mg per kg per day twice a day; maximum of 30 mg per day; (subjects taking concomitant STP will receive 0.5 mg per kg per day)	https://clinicaltrials.gov/study/NCT05064878
STK-001	ASOs to specifically increase productive mRNA levels, leading to optimal protein expression	B	No	Phase I and II trial in paediatric patients with DS (MONARCH study -NCT04442295), active, not recruiting USA Phase I and II trial in paediatric patients with DS (ADMIRAL study - EudraCT Number 2020-006016-24, active, not recruiting UK	Multiple ascending doses administered as an intrathecal injection. (10mg, 20mg,30mg, 45mg and 70mg)	https://clinicaltrials.gov/study/NCT04442295 https://clinicaltrials.gov/study/NCT04740476
Quinidine	Potassium sodium-activated channel subfamily T member 1 (KCNT1) current blocker	C	Yes, not for epilepsy (repurposing drug)	EIFMS and other refractory DEE (> 27 clinical studies, >82 treated patients)	Range from 2 mg per kg per day to 126 mg per kg per day.	n.a.
Fluoxetine	Potassium channel blocker	D	Yes, not for epilepsy (repurposing drug)	*KCNC1*-related DEE (single cases) *KCNT2*- related DEE (*in vitro* studies)	n.a.	n.a.

Radiprodil	Negative allosteric modulator of the NMDA subtype of glutamate receptor, with selectivity for receptor complexes containing the GluN2B subunit.	C	No	Phase Ib study in three infants with West syndrome, resistant to combination therapy with vigabatrin and prednisolone Phase 1B trial RAD-GRIN-101 2022-000317-14 (EudraCT Number) in *GRIN*-related disorder in children with a Gain-of-Function (GoF) genetic variant (*GRIN1, GRIN2A, GRIN2B, GRIN2D*)	0.04 mg per kg per day (Low), 0.10 mg per kg per day (Medium), and 0.21 mg per kg per day (High). 0,5 mg per kg per day- 2 mg per kg per day	https://clinicaltrials.gov/study/NCT05818943
PRAX-562	Inhibition of $NaV_{1.6}$ persistent sodium current (I_{Na}).	D	No	Phase II EMBOLD study in early-onset *SCN2A*-DEE and *SCN8A*-DEE (GoF variants)	0.5mg per kg per day	https://clinicaltrials.gov/study/NCT05818553
BHV-7000	Kv7.2 and Kv7.3 activator	D	No	Phase II and III study in adults with refractory focal onset epilepsy (future possible application in *KCNQ2-KCNQ3* related DEEs)	25-50 mg per day	https://clinicaltrials.gov/study/NCT06132893
Clemizole	First-generation antihistamine drug, with selectivity for the H1 subtype of histamine receptor, but reduce electrographic seizure activity via modulation of serotonergic rather than histaminergic signalling	B	No	Phase II study in patients with DS (NCT04462770)	n.a.	https://clinicaltrials.gov/study/NCT04462770

mTOR = mechanistic target of rapamycin; FDA = Food and Drug Administration; EMA = European Medicine Agency; TSC = Tuberous Sclerosis Complex; IS = infantile spasms; n.a. = not available; DS = Dravet Syndrome, LGS = Lennox Gastaut Syndrome, EIFMS = Epilepsy of Infancy with Migrating Focal Seizures; CDD = CDKL5 Deficiency Disorders; DEE = Developmental and epileptic encephalopathies 5-HT2C = 5-hydroxytryptamine 2C; NMDA = N-methyl-D-aspartate; GABAA = γ-Aminobutyric acid type A; GABA-T = gamma-amino-butyric acid transaminase; ASOs = Antisense oligonucleotides; GoF = Gain of Function; STP = stiripentol.