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SPECIAL REPORT

WONOEP appraisal: Targeted therapy development for early onset epilepsies

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Abstract

The early onset epilepsies encompass a heterogeneous group of disorders, some of which result in drug-resistant seizures, developmental delay, psychiatric comorbidities, and sudden death. Advancement in the widespread use of targeted gene panels as well as genome and exome sequencing has facilitated the identification of different causative genes in a subset of these patients. The ability to recognize the genetic basis of early onset epilepsies continues to improve, with de novo coding variants accounting for most of the genetic etiologies identified. Although current disease-specific and disease-modifying therapies remain limited, novel precision medicine approaches, such as small molecules, cell therapy, and other forms of genetic therapies for early onset epilepsies, have created excitement among researchers, clinicians, and caregivers. Here, we summarize the main findings of presentations and discussions on novel therapeutic strategies for targeted treatment of early onset epilepsies that occurred during the Workshop on Neurobiology of Epilepsy (WONOEP XVI, Talloires, France, July 2022). The presentations discussed the use of chloride transporter inhibitors for neonatal seizures, targeting orexinergic signaling for childhood absence epilepsy, targeting energy metabolism in Dravet syndrome, and the role of cannabinoid receptor type 2, reversible acetylcholinesterase inhibitors, cell therapies, and RNA-based therapies in early life epilepsies.

KEYWORDS

acetylcholinesterase, bumetanide, cannabidiol, cannabinoid receptor type 2, developmental and epileptic encephalopathies, interneuron, miRNA, orexin

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For affiliations refer to page 335.

1 | INTRODUCTION

The incidence of epilepsy ranges between 41 and 187/100 000 children and peaks within the first year of life.[1] The etiology of early life epilepsy is heterogeneous.[2–5] The majority of childhood and early onset epilepsies are due to genetic, structural/metabolic causes or unknown etiologies, posing a challenge in developing etiology-specific treatments, as the cause is either multifactorial or unknown.[3–6] Structural–metabolic etiologies are also more likely to be associated with resistance to available antiseizure medications (ASMs). Approximately 10%–22% of epilepsy cases have an identifiable genetic etiology,[1,7–9] and Mendelian inheritance constitutes 1%[6,10] of these cases.[1,9] Even though some of the early onset epilepsy syndromes (epilepsy onset before the age of 12 months) may have favorable prognoses, others, like the developmental and epileptic encephalopathies (DEEs), result in drug-resistant seizures (i.e., drug-resistant epilepsy),[9,11] developmental delay, risk of premature mortality in epilepsy, intellectual disability, and other neurological symptoms.[5,12] Neonatal seizures are a particular challenge given the significant age and sex-specific differences between the newborn brain and the adult brain (molecular, cellular, structural, metabolic, connectivity), the different effects of ASMs in the developing brain, and the large spectrum of etiologies triggering early life seizures.[11–14] Despite the development of more than 20 ASMs over the past 30 years, the treatment of early onset epilepsies remains only symptomatic, and in many epilepsy cases, neither seizures nor comorbidities can be controlled with ASMs.[12–14] The lack of disease-specific and disease-modifying therapies are critical issues in early onset epilepsies.

Recent technological advances, including targeted gene panels and next generation exome and genome sequencing, have identified a wide range of genetic variants associated with the development of early onset epilepsies.[6–8,15–18] The increased accessibility of these technologies has facilitated the diagnosis of new cases and improved the mapping of genetic profiles, and, in turn, a growing number of causative genes have been identified.[15–17] These ever-increasing numbers of identified genes allow therapies to target a clearly identified pathogenic cause, the genetic variant and its direct functional effects, in precision medicine efforts. However, in many instances, even when the genetic cause is known, treatments are still elusive, as the consequences of the mutation remain unclear.[19] The development of treatments requires models that can recapitulate epilepsy patients' phenotype, raise hypotheses, test novel treatments, and expand our understanding of the epileptogenesis process in early onset epilepsies. For example, novel precision medicine approaches, such as gene and cellular therapy, have created excitement within the epilepsy community toward effective treatments of early onset epilepsies. Furthermore, a stubborn gap in epilepsy therapy requires new platforms for the development of treatments for drug-resistant seizures.[20] During the Workshop on Neurobiology of Epilepsy (WONOEP XVI), organized in 2022 by the Neurobiology Commission of the International League Against Epilepsy, one of the main areas for discussion was novel therapeutic strategies for targeted treatment of early onset epilepsies. This article was conceptualized through WONOEP, and we summarize the presentations and relevant discussion of research centered around novel therapeutic mechanisms, cell therapy, and RNA therapy aimed to rectify the pathophysiological mechanisms leading to the development of early onset seizures and epilepsies.

Key points

- Promising repurposed therapy for early onset epilepsy acts on novel mechanisms and pathways.
- CB2R regulates both postsynaptic function and neuroinflammation.
- Correction of dysregulated metabolic pathways can be therapeutic in Dravet syndrome.
- Interneuron cell transplantation is a novel mechanism to modulate GABA function in early onset epilepsies.
- miR-335 shows promise for regulating sodium channels (*Scn*) relevant to early onset epilepsies.

2 | SMALL MOLECULES TO TARGET NOVEL PROCESSES IN EARLY ONSET EPILEPSIES

2.1 | Restoring metabolism homeostasis in DEEs

Altered metabolism is an emerging feature of acquired and/or focal epilepsies, that has not been systematically investigated in genetic early onset epilepsies.[21] Dravet syndrome (DS), a DEE with poor prognosis,[5] is associated with de novo mutations in the *SCN1A* gene, encoding the alpha subunit $Na_v1.1$ of voltage-activated sodium channels.[22–25] Mutations in *SCN1A* are associated with DS and genetic epilepsy with febrile seizures plus, and numerous mouse models exist to study the effect of *Scn1a* mutations.[26] DS patients suffer from drug-resistant early onset seizures and debilitating comorbidities. The role of energy metabolism in genetic epilepsies such as DS

remains relatively unexplored.[27,28] Zebrafish research has been instrumental in recent advances in understanding and treating DS.[29] The translational zebrafish model (*scn1lab*), exhibits key clinical characteristics of DS and shows metabolic deficits accompanied by downregulation of the gluconeogenesis genes, *pck1* and *pck2*. Using a novel technique to simultaneously measure glycolysis and mitochondrial respiration in real time, a recent study has shown abnormal metabolism in the *scn1lab* model of DS and its rescue by the ketogenic diet.[30] Furthermore, a metabolism-based small library screen identified compounds that increased gluconeogenesis via upregulation of *pck1* gene expression in *scn1lab* larvae. One of the compounds identified, PK11195, a *pck1* activator and a translocator protein (TSPO) ligand, normalized dysregulated glucose levels, metabolic deficits, and TSPO expression and significantly decreased electroencephalographic seizures in mutant *scn1lab* larvae. Moreover, inhibition of *pck1* in wild-type larvae mimicked metabolic and behavioral deficits observed in *scn1lab* mutants. Together, the data suggest that correcting dysregulated metabolic pathways can play a therapeutic role in DS.

2.2 | Endocannabinoid system modulation in early onset epilepsies

The roles of the cannabinoid receptor type 1 (CB1R) and cannabinoid receptor type 2 (CB2R) in the central nervous system (CNS) have been widely documented in the literature.[31] CB1R was the first cannabinoid receptor described, is widely present in neurons, and is the major mediator of the psychoactive effects of the plant *Cannabis sativa* and its derivatives.[31,32]

CB2R was initially described as an immunomodulator of white blood cells.[33] However, evidence has described its expression and involvement in the CNS,[34] particularly in the neuronal postsynaptic cell body regulating neuronal function[35] and in modulating neuroinflammatory responses.[31] Importantly, CB2R activation lacks the psychoactive effects described for CB1R. Moreover, brain CB2R is expressed at low levels under physiological conditions, but the expression is markedly increased after brain insults, including traumatic brain injury[36] and stroke.[31,37] Overall, the inducible feature of CB2R has the potential to serve as an epilepsy-associated target, amenable to pharmacological manipulation without unwanted psychoactive side effects.

Recent evidence shows that CB2R also has modulatory effects on inflammation, microglia, and brain-infiltrating monocytes.[38] A recent review highlighted several studies that observed a reduction in proinflammatory cytokines and downstream pathways, and reduced inducible nitric oxide synthase and oxidative molecules related to the M1 polarization state, as well as increased expression of microglia M2-related proteins, including interleukin-10, arginase-1, and CD206, following CB2R activation.[31]

Infiltrating monocytes integrate into the brain parenchyma to differentiate into monocyte–macrophages.[31,39] However, their role during epileptogenesis is poorly understood and difficult to unravel from that of microglia. The identification of specific monocyte–macrophage markers and the development of flow cytometry techniques make it possible to isolate, quantify, and characterize the infiltrating monocyte–macrophage's inflammatory state.[40] Efforts must be maintained to identify other specific markers, particularly in other species, such as rats and humans, in which isolation of this cell population is still challenging. The specific involvement of monocyte–macrophages over microglia in both seizures and associated memory deficits has been recently highlighted in the Theiler murine encephalomyelitis virus model of epilepsy.[41] Further studies are needed to better understand the contribution of these cells to neuroinflammation and its consequences on the pathophysiology of other epilepsy syndromes. To further expand the potential neuroprotective effects of CB2R, a recent study demonstrated that CB2R knockout mice are more susceptible to induced seizures by pentylenetetrazole (PTZ), and that CB2R antagonism increased susceptibility to PTZ-induced seizures in wild-type mice.[42,43] Moreover, *Scn1a* mutant mice displayed increased seizure resistance after the administration of a positive allosteric modulator of CB2R.[42,43] Moreover, recent preclinical evidence suggests that modulation of the endocannabinoid system may offer a therapeutic strategy to reduce seizures and improve cognitive comorbidities.[44] GAO-3-02, a derivative of synaptamide that targets CB2R,[44] is currently in preclinical development and could represent a promising approach for improving outcomes in early onset epilepsies. Overall, mounting evidence suggests that targeting CB2R might provide a therapeutic target for treating early onset epilepsies.

2.3 | Reversible acetylcholinesterase inhibitors as potential treatments for early onset epilepsies

Acetylcholine (ACh) is the main excitatory neurotransmitter in the peripheral nervous system and is a major regulator of excitability in the forebrain.[45] As with most neuromodulators, a fine balance is required to maintain neuronal excitability. Overactivation of the nicotinic and muscarinic receptors can result in seizures and epilepsy.[45] One of the most well-characterized models of focal onset epilepsy uses pilocarpine, a potent

muscarinic ACh receptor agonist, to induce status epilepticus and focal onset epilepsy.[45] Moreover, ACh nicotinic receptor stimulation has been used to induce kindled seizures in mice.[46] Clinically, gain of function mutations in ACh receptors have been reported in specific genetic syndromes such as autosomal dominant sleep-related hypermotor (hyperkinetic) epilepsy (previously known as autosomal dominant nocturnal frontal lobe epilepsy and autosomal dominant sleep-related hypermotor epilepsy),[2] and have been implicated in the development of seizures in Rett syndrome.[45]

In contrast, there is evidence that a moderate increase in ACh receptor activity may be beneficial.[47] Huperzine A (Hup A) and donepezil are reversible acetylcholinesterase (AChE) inhibitors previously investigated in dementia and Alzheimer disease. A recent study showed the protective effects of Hup A in *Scn1a* and *Scn8a* mouse models. Interestingly, Hup A provided robust protection against 6 Hz- and PTZ-induced seizures in two *Scn1a* mouse models and in *Scn8a* R1620L (RL/+) mutants.[48,49] The RL/+ mutants harbor the human R1620L mutation and were previously shown to exhibit increased seizure susceptibility, spontaneous seizures, and a range of behavioral abnormalities, including hyperactivity, learning and memory impairment, and social behavior deficits.[48] Hup A-mediated seizure protection was shown to require muscarinic receptors and in part, the γ-aminobutyric acid $(GABA)_A$ receptor.[50] Similarly, donepezil administration (an US Food and Drug Administration-approved reversible AChE inhibitor) increased seizure resistance in *Scn1a* mutants.[51] Although further studies are needed, modulation of ACh receptor signaling demonstrates treatment potential for early onset epilepsies.

2.4 | Altering chloride transport to improve treatment of phenobarbital-resistant neonatal seizures

Phenobarbital increases the amount of time $GABA_A$ receptor chloride channels are open by interacting with the $GABA_A$ receptor subunits, typically resulting in hyperpolarization of the postsynaptic mature neuron. However, in early developmental stages (i.e., in immature neurons), the activation of $GABA_A$ receptors exerts paradoxical depolarization.[52] The proper timing of the GABA "switch" from depolarizing to hyperpolarizing is critical for brain development and is also sex-specific.[53–56] In animal models, the switch in $GABA_A$ receptor signaling polarity has been shown to be biphasic. The first phase occurs shortly before delivery, where there is a transient reduction in the intracellular chloride concentration and a depolarizing-to-inhibitory switch of $GABA_A$ receptor signaling.[57] The second phase is a progressive and permanent change in GABA-activated membrane polarity that starts soon after birth and is completed during the early postnatal weeks, following cell type-, region-, and sex-specific patterns.[53–56,58,59]

Phenobarbital has been used to treat neonatal seizures for many decades.[18] The anticonvulsant effect of phenobarbital has unique features in neonates.[60] For example, patients whose electroclinical seizures are stopped by phenobarbital still have ongoing electrographic seizures, a phenomenon called uncoupling.[61,62] Recent studies have demonstrated that phenobarbital was very effective in treating patients with low seizure burden[60] but is much less effective at treating severe neonatal seizures.[63,64] Whether there is critical seizure burden that determines treatment efficacy has not yet been resolved.[65,66]

One of the hypotheses that can explain the limitation of the phenobarbital response arises from the ionic forces that drive the inhibitory membrane currents that are gated by GABA and enhanced by phenobarbital. Seizures are associated with increased GABA receptor signaling, which in turn can lead to the accumulation of chloride, the principal permeant ion, on the cytoplasmic side of the channel. Experimental studies have demonstrated accumulation of cytoplasmic chloride during ongoing seizures in perinatal brain.[67–69] This chloride accumulation can be sufficient to reverse the direction of chloride flux when the $GABA_A$ receptor channel is open, resulting in ineffective inhibition, and potentially even excitation, of neurons.[67] Under these conditions, increasing $GABA_A$ receptor channel opening with phenobarbital will not be an effective antiseizure strategy. Ideally, the enhancement of electroneutral chloride export might be useful to address this problem, but such therapies are still being developed and require the expression of potassium chloride cotransporter 2 (KCC2). In the meantime, another study tested whether blocking sodium potassium chloride transporter 1 (NKCC1), which is more active in immature than in mature neurons, would improve the chloride balance, $GABA_A$ receptor-mediated inhibition, and the effect of phenobarbital. NKCC1 is also expressed at the blood–brain-barrier[70] and is thought to serve as a mechanism to move chloride from the blood to the brain, where it may accumulate in neurons as described above and contribute to phenobarbital failure.

Blocking NKCC1 with the loop diuretic bumetanide effectively suppressed epileptiform activity in hippocampal slices in vitro and reduced kainic acid-induced seizures in neonatal rats.[71] A preliminary human dose-finding study failed to demonstrate efficacy, but that was an uncontrolled

study in which much of the efficacy signal was derived from patients who did not have seizures during the baseline period, that is, patients for whom NKCC1 inhibition could not be effective.[69,72] To determine whether bumetanide would also be helpful to reduce seizures in human neonates, a phase 2 double-blind randomized controlled trial tested bumetanide in neonates who had failed to respond to phenobarbital. The neonates were randomized to an increased dose of phenobarbital and placebo, or phenobarbital plus bumetanide. Although phase 2 trials are not powered to demonstrate efficacy, Soul and colleagues found that the higher doses of bumetanide were effective, particularly for those with high seizure burdens (i.e., those for whom phenobarbital is less effective).[73] To ascertain efficacy, a larger phase 3 trial will be necessary. Along those lines, NPT 2042, a bumetanide analogue,[74] is currently undergoing a safety and pharmacokinetic trial (ClinicalTrials.gov ID NCT05503511). However, details on its structure and pharmacological properties are not yet available.[74]

2.5 | Targeting the orexin system to modulate absence seizures

Absence seizures are the predominant seizure type of certain patients with idiopathic generalized epilepsy (IGE) syndromes.[5,75] Initiated by cortical layer neurons, spike-and-wave discharges (SWDs) result from paroxysmal oscillations in the corticothalamocortical network and are also associated with vigilance control and sleep–wake states.[76]

SWDs are most numerous during slow-wave sleep and non-rapid eye movement sleep and least frequent during rapid eye movement sleep, both in people with IGE and absence seizures, and in genetic models such as the Genetic Absence Epilepsy Rat from Strasbourg (GAERS) and Wistar Albino Glaxo Rat of Rijswijk (WAG/Rij) rat strains.[77–79] Importantly, both SWDs and sleep spindles are generated in the same corticothalamocortical networks.[80,81] The orexin system is one of the major sleep–wake cycle regulators in the brain.[80] The two main receptors for orexin, orexin-1 and orexin-2, are activated primarily by two orexin neuropeptide isoforms, orexin A and B, respectively.[82] Orexin-producing neurons are mainly localized in the lateral hypothalamus and project to different brain areas involved in the sleep–wake cycle.[83] Importantly, receptors are >95% homologous between humans and rats and are widely expressed in the thalamus and the neocortex.[82,84] Under physiological conditions, orexin peptides may excite thalamic neurons directly via the orexin receptors or indirectly by activating wake-promoting pathways projecting to the thalamus, including T-type calcium channels.[85] Orexin-induced depolarization may facilitate T-type calcium channel inactivation in thalamic neurons, thereby restraining the development of SWDs. Specific inhibition of T-channels is a promising disease-modifying therapy to prevent the development and progression of focal and generalized epilepsies in animal models, but clinical support for this is currently lacking.[86–88] Hence, it is reasonable to hypothesize that the orexinergic system may have the potential to suppress absence seizures via its T-type calcium channel modulation.

The evidence of orexin receptor modulation of SWD is mainly derived from models of childhood onset epilepsy. In the WAG/Rij model of IGE with absence seizures,[89] orexin-1 receptor protein expression was decreased in the thalamus and somatosensory cortex of epileptic adult WAG/Rij rats in contrast to age-matched Wistar nonepileptic controls.[80] However, no significant changes were observed before the development of SWDs in WAG/Rij compared to Wistar rats, suggesting a correlation between the presence of absence seizures and orexin-1 receptor changes.[80] Furthermore, the administration of a selective orexin-2 receptor agonist decreased the incidence of SWDs in adult GAERS, which was correlated with the reduced levels of orexin-2 receptor protein in the somatosensory cortex and thalamus compared to age-matched nonepileptic Wistar rats.[90] An interesting finding in hippocampal slices from mice was that orexin-A administration decreased the duration and amplitude of epileptiform discharges induced by the $GABA_A$ antagonist bicuculline, suggesting that orexin-A may have antiseizure effects.[91] These findings suggest that orexins may modulate neurotransmission in the hippocampus, potentially by modulation of GABAergic and glutamatergic systems. Similarly, studies have reported modulatory actions of orexins on neurotransmission in different thalamic nuclei, which are critical for the development of SWDs in animal models.[82,85,92,93] To date, no studies have investigated the role of orexin-1 and orexin-2 receptors in early onset epilepsies. Further in-depth analysis of the orexin system may provide interesting insight into the possible functional significance in the pathophysiology of the development of typical and atypical absence seizures.

The translational relevance of the findings on orexin should be considered cautiously, however. The onset of epilepsy in the GAERS model is at approximately 3 weeks of age,[94] and in the WAG/Rij, 3–4 months,[95] in contrast to monogenic mouse models of absence seizures, where spike–wave seizures begin in the second postnatal week.[96,97] However, some underlying mechanisms, including T-current, may be shared across all these models.[98]

Thus, it remains to be demonstrated that orexin is a candidate treatment for early onset epilepsies.

3 | POTENTIAL OF CELL THERAPY TO REPAIR PATHOLOGICAL SUBSTRATE OF EARLY ONSET EPILEPSIES

Cell therapy aims to replace, repair, or enhance the biological function of damaged tissue, in this case, a brain region, by either transplanting cells or modulating specific cell populations in the target area to ameliorate the lost function.[99] In the case of epilepsy, transplanting cells into the seizure onset zone aims to replace injured or dysfunctional neurons.[99] Moreover, cell therapy could supplement neuropeptides that modulate the microenvironment and potentially attenuate seizures.[100–102]

Several studies have proved enhanced inhibitory synaptic transmission as a successful mechanism of action to exert antiseizure effects that are particularly relevant for early onset epilepsies.[99,103,104] A reduction in seizure frequency and severity has been achieved in animal models by transplanting allogeneic mouse medial ganglionic eminence (MGE)-derived GABAergic progenitor cells and human pluripotent stem cell-derived GABAergic progenitors.[103–106] Currently, human embryonic stem cell-derived MGE-GABAergic interneurons are being tested in a clinical trial to reduce seizures in drug-resistant temporal lobe epilepsy (trial ID NCT05135091).

Few studies have used cell therapy to inhibit dysfunction early in development. Baraban and colleagues showed a reduction of seizures after allogenic mouse MGE progenitor transplantation into the early postnatal neocortex of *Kv1.1* mutant mice, but the cell transplantation occurred before seizure onset.[107] On the other hand, transplantation of allogenic mouse neuronal precursors into the medial prefrontal cortex improved behavioral deficits in a maternal immune activation model of autism spectrum disorder[108] and in *Pten* mutant mice.[109] It is important to note, however, that no spontaneous seizures are seen in the abovementioned mouse models.

Most of the work using cell therapy to treat epilepsy has been performed in models of focal epilepsy and temporal lobe epilepsy; hence, the suitability and effects to mitigate seizure burden for early onset epilepsies remain to be determined. For example, changes in NKCC1 and KCC2 expression during development lead to GABA polarity shift due to variations in chloride homeostasis.[99] Therefore, when designing a cell therapy for early onset epilepsy, it is crucial to consider that physiological developmental changes may affect the survival, integration, and phenotype specification of the transplanted cells, as well as how the underlying genetic causative variant could also exert an effect on the grafted neurons.

4 | RNA THERAPY TO REGULATE SODIUM CHANNELS IN DEEs

Recent single-cell and multiomic analyses of brain tissue from patients and in animal models of treatment-resistant epilepsy reveal complex and multifactorial pathophysiology.[110–113] This suggests multitargeting approaches may be needed to achieve disease-modifying effects.[113] MicroRNAs have emerged as potential targets in this regard.[114] These small noncoding RNAs each posttranscriptionally suppress multiple transcripts in the same as well as different pathways. In so doing, they fine-tune gene expression patterns throughout the body and extensively in the brain. Various microRNAs have been identified that control neuronal structure and function, gliosis, metabolism, inflammation, and cell death.[115] Each phase of the epileptogenesis process is associated with dysregulation of sets of microRNAs,[115] and in vivo studies in rodents suggest targeting some of these can reduce evoked and spontaneous seizures.[114]

Recent studies show that ion channels associated with channelopathies are also under microRNA control.[116,117] To identify novel microRNAs relevant to epilepsy, recent work has evaluated microRNA expression changes across three different microRNA datasets: the hippocampus of rats after perforant pathway stimulation, the hippocampus of mice after a 5-day course of cannabidiol, and plasma samples from patients undergoing presurgical evaluation. These converged on a single microRNA, miR-335.[118] Importantly, the predicted targets of miR-335 are critical genes that regulate excitability, including a set of sodium channels. Modulation of sodium channels could have applications in different early onset epilepsies and DEEs. In vivo inhibition of miR-335 using antisense oligonucleotides (microRNA inhibitors) resulted in upregulation of select sodium channel transcripts and increased excitability in a brain slice model and the PTZ model of evoked seizures in mice.[118] Blocking miR-335 also increased expression of certain sodium channel transcripts in human neurons, including *SCN1A*. In contrast, overexpression of miR-335 via adeno-associated virus serotype 9 reduced levels of several targets and protected against PTZ-induced seizures and mortality.[118] These studies demonstrate a role for miR-335 in stabilizing neuronal network excitability and an additional approach for seizure control.[118] It is unknown, however, whether the overexpression of miR-335 can protect against spontaneous seizures or whether there is synergism with sodium channel-blocking drugs. Options worth considering are to direct the microRNA to

specific cell types and to design studies to explore their effects in human induced pluripotent stem cell models, organoids, or resected brain tissue. Likewise, microRNA can be packaged in exosomes and directly transmitted into the CNS via nasal delivery, potentially avoiding systemic effects.[119,120] It may also be possible to develop a more precise microRNA targeting strategy that would reduce the number of targets altered by modulating this microRNA. Target site blockers are based on adapted microRNA-targeting oligonucleotides that are designed to disrupt only the interaction between the microRNA and specific transcripts. Future studies might investigate whether a target site blocker can be designed to interrupt the specific effects of miR-335 on *Scn1a* or *Scn2a* while sparing other miR-335 targets. These and other studies would help to ensure the safety and specificity of microRNA-based therapies.

5 | DISCUSSION AND FUTURE DIRECTIONS

The identification of causative genes has been a tremendous advantage for generating specific genetic animal models that facilitate hypothesis testing and a better understanding of the molecular determinants leading to the development of early onset epilepsies.[52,121] Given the phenotypic pleiotropy associated with genetic variants and their direct functional effects, a precision medicine approach targeting the underlying pathology (i.e., specific genetic variant) and pathological processes can potentially treat the overall cause and improve the symptomatology for early onset epilepsy patients.[19]

However, the initial genetic variant can often be the starting point of a pathophysiological series of events (Figure 1), because the perturbed biological system can respond in multiple ways by developing further complex modifications, some that aggravate the phenotype (propathological responses), others that counteract pathological dysfunctions (homeostatic responses).[123] These responses secondary to the precipitating genetic variant can be important for determining the mature phenotype of the disease. Thus, identifying the secondary responses to the genetic variant is important for unveiling the complex pathophysiological mechanisms of early onset epilepsies and developing effective targeted therapeutic approaches.[124] For instance, several secondary modifications have been identified in models of DEEs, in particular DS.[125] An example is the hyperexcitability of some glutamatergic neurons during late disease stages upon a protocol of induced seizures that triggers a DS-like phenotype in the *Scn1a*$^{RH/+}$ knockin mouse model,[126] which in early stages shows only hypoexcitability of GABAergic neurons.[12,127] Therefore, identifying secondary modifications can disclose novel druggable targets to inhibit propathological responses or enhance homeostatic ones.

The clinical success of disease-modifying therapies in epilepsy depends on a comprehensive understanding of the pathophysiological mechanisms that goes beyond single-gene variant etiologies.[110,113] This knowledge should include phenotypic features that are age-specific and consider other non-epilepsy-related comorbidities. Unraveling these biological mechanisms will expand the benefits of a therapeutic intervention beyond the simple seizure burden, including functional abilities and overall quality of life.

Altogether, the work presented at the WONOEP XVI meeting suggests a rich and diverse set of approaches to treating DEEs and other early onset epilepsies, spanning from gene and cell therapy to small molecules.

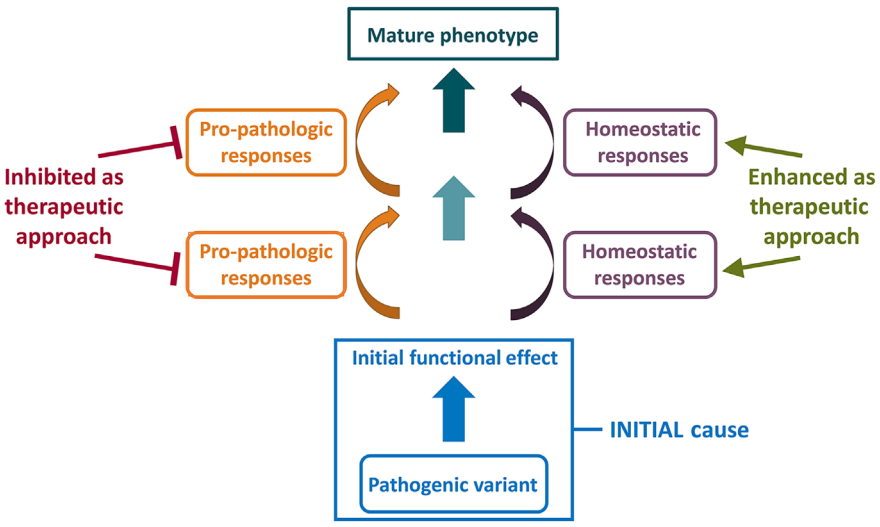

FIGURE 1 Propathological and homeostatic responses. This diagram shows how propathological and homeostatic responses secondary to the precipitating pathogenic genetic variant may lead to a mature disease phenotype.[122] Moreover, both propathological and homeostatic responses are amenable targets for modification to improve outcomes in early onset epilepsies.

AUTHOR CONTRIBUTIONS

Pablo M. Casillas-Espinosa led manuscript drafting. Pablo M. Casillas-Espinosa, Jennifer C. Wong, Wanda Grabon, Ana Gonzalez-Ramos, Massimo Mantegazza, Nihan Carcak Yilmaz, Manisha Patel, Kevin Staley, David C. Henshall, and Aristea S. Galanopoulou contributed to sections of the manuscript and reviewed and edited the manuscript. Raman Sankar, Özlem Akman, Stéphanie Baulac, Terence J. O'Brien, Stéphane Auvin, Ganna Balagura, Adam L. Numis, and Jeffrey L. Noebels contributed to manuscript writing and critical review/editing.

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CONFLICT OF INTEREST STATEMENT

D.C.H. reports European Patent Application No. EP21198390.3 "Modulation of MicroRNA-335-5p for the Treatment of Sodium Channelopathies." D.C.H. and RCSI have filed a patent application based on modifying miR-335 to treat seizure-related disorders. Outside the submitted work, P.M.C.-E. has received research grants from CSIRO, Data61, Supernus Pharmaceuticals, Praxis, Eisai, and Kaoskey. P.M.C.-E. declares no conflict of interest with the current work. A.S.G. is editor-in-chief of *Epilepsia Open* and associate editor of *Neurobiology of Disease*, and receives royalties from Elsevier (publications, journal editorial board participation), Wolters Kluwer, and MedLink (publications). S.A. is deputy editor for *Epilepsia*. He has served as a consultant for or received honoraria for lectures from Angelini Pharma, Biocodex, BioMarin, Encoded, Eisai, GRIN Therapeutics, Jazz Pharmaceutics, Neuraxpharm, Nutricia, Orion, UCB Pharma, Xenon, and Zogenix. He has been an investigator for clinical trials for Eisai, Marinus, Proveca, UCB Pharma, Xenon, and Zogenix. R.S. has served as a consultant and/or speaker for which he has received honoraria from Biocodex, Eisai, BioMarin, Jazz Pharmaceuticals, Neurelis, Ovid, SK Life Science, UCB Pharma, and Zogenix. T.J.O.'s institution has received research funding from Eisai, UCB Pharma, LivaNova, ES Therapeutics, and Kinoxis Therapeutics. He has also received competitive grant funding from the NHMRC, MRFF, NINDS, and DoD. None of the other authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

DATA AVAILABILITY STATEMENT

This is an appraisal of WONOEP; no new data were generated.

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