Gene therapy for epilepsy: From *SCN1A* to broadened therapeutic horizons (Review)

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Abstract. Genetic epilepsy, for which the molecular mechanisms are relatively well defined in numerous monogenic forms, represents an attractive model for the transition from symptomatic seizure control to mechanism-driven correction. However, gene therapy strategies differ in their mechanisms of action, adaptability to specific mutations and translational maturity, making rational selection of interventions based on variant-specific functional consequences an important clinical challenge. The present review examined five nucleic acid-based therapeutic strategies, namely gene supplementation, enhancement of endogenous gene expression, gene silencing, DNA editing and RNA editing, for major causative genes, using mutation-specific functional consequences [loss-of-function (LOF) vs. gain-of-function] as the primary framework for therapeutic decision-making. The mechanisms and suitability of each approach are analysed, and their performance in terms of cell-type specificity, delivery vectors and safety is evaluated by integrating evidence from animal models, induced pluripotent stem cell-derived neurons and organoids and clinical trials. The present review also summarised key translational barriers, including vector cargo constraints, blood-brain barrier penetration, off-target and immune-associated risks, therapeutic dosing windows and the timing of developmental intervention. Antisense oligonucleotides and regulatory adeno-associated viral approaches, supported by mature delivery platforms and manageable safety profiles, have progressed furthest towards clinical validation. Endogenous gene activation strategies, such as CRISPR activation, offer a gene replacement-free approach for LOF disorders but remain preclinical. DNA and RNA editing, while potentially capable of durable disease modification, require further evidence on delivery efficiency and long-term homeostatic safety. Gene therapy for genetic epilepsy is undergoing a key transition from proof-of-concept studies to individualised clinical application. Future progress will depend on integrating personalised intervention design, multi-target therapeutic combinations, artificial intelligence-assisted sequence optimisation and standardised translational models, with the potential to drive a paradigm shift from seizure suppression to mechanistic correction.

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1. Introduction

Epilepsy is a chronic neurological disorder characterised by abnormal discharges within neuronal networks of the brain and affects >50 million individuals worldwide. Although anti-seizure medications can control seizures in a substantial proportion of patients, ~1/3 fail to achieve sustained seizure freedom and develop drug-resistant epilepsy, which is associated with increased risks of injury, cognitive impairment and sudden unexpected death in epilepsy (1,2). In genetic epilepsies, conventional therapies, such as sodium channel blockers or GABA enhancers, primarily modulate neuronal excitability and do not directly address the underlying molecular aetiology; therefore, developing aetiology-directed precision therapies is important (1).

In paediatric populations, the consequences of genetic epilepsy extend beyond seizure burden. Early-onset seizures

disrupt key periods of neurodevelopment, impairing synaptic pruning, myelination and network maturation (3). Children with severe genetic epilepsies, such as Dravet syndrome (DS) and sodium voltage-gated channel α subunit 8 (*SCN8A*) encephalopathy, frequently experience developmental regression, cognitive impairment and motor dysfunction that progressively worsen over time (4,5). Globally, ~6.1 million children <14 years are living with epilepsy, with an estimated 1.23 million new cases diagnosed annually, with the highest incidence occurring in the first year of life (6). This substantial disease burden, reflected in >3.5 million disability-adjusted life years and nearly 18,200 mortalities per year, underscores the key importance of early intervention (6). The combined effects of recurrent seizures, medication-associated adverse effects and the underlying molecular pathology contribute to a progressive neurodevelopmental course that becomes increasingly difficult to reverse when intervention is delayed (7). This developmental urgency highlights the need for aetiologically directed therapies that can be implemented during early childhood to prevent or attenuate neurodevelopmental deterioration (8).

As several genetic epilepsies have a relatively well-defined molecular basis, they are considered an ideal model for precision medicine and aetiological intervention. Studies have demonstrated that mutations in key genes, including sodium voltage-gated channel α subunit 1 (*SCN1A*), sodium voltage-gated channel α subunit 2 (*SCN2A*), *SCN8A*, potassium voltage-gated channel subfamily Q member 2/3 (*KCNQ2/3*), tuberous sclerosis complex (*TSC*)1/2, glutamate ionotropic receptor N-methyl-D-aspartate (*NMDA*) type subunit 2A (*GRIN2A*) and syntaxin binding protein 1 (*STXBP1*), can directly disrupt the excitatory-inhibitory balance of neuronal networks by altering ion-channel function, synaptic transmission or the homeostasis of cellular signalling pathways (9-16). By contrast, current anti-seizure medications largely suppress neuronal excitability and therefore cannot correct specific molecular defects. In certain genetic epilepsy subtypes, these agents may even exacerbate disease manifestations. For example, in *SCN1A*-associated DS, sodium-channel blockers often worsen clinical symptoms (17). This symptom-oriented rather than cause-directed therapeutic paradigm, together with the adverse effects associated with existing medications, remains a major challenge in the clinical management of epilepsy.

In clinical practice, determining whether a newly identified variant confers loss-of-function (LOF) or gain-of-function (GOF) effects is an important first step in selecting an appropriate therapeutic strategy. This classification relies on a multi-layered approach. First, variant type and location should be considered. Nonsense, frameshift and splice-site variants typically result in LOF through protein truncation or degradation, whereas missense variants affecting functionally key domains, such as voltage-sensing or pore-forming regions, often produce GOF effects. Second, family segregation and inheritance patterns may reveal pathogenic mechanisms, including haploinsufficiency and dominant-negative effects. Third, *in vitro* functional studies, including electrophysiological recordings in heterologous expression systems and patient-derived induced pluripotent stem cell (iPSC) neurons, can directly assess alterations in channel kinetics, conductance and gating properties. Fourth, predictive computational tools, including artificial intelligence (AI)-based algorithms such as Combined Annotation-Dependent Depletion, Rare Exome Variant Ensemble Learner and AlphaMissense, can estimate variant pathogenicity and functional impact, although experimental validation remains key. Finally, genotype-phenotype correlations may offer additional evidence. For instance, sodium-channel blockers typically worsen *SCN1A* LOF-associated disease but may provide clinical benefit in *SCN8A* GOF-associated disorders. Collectively, this integrative framework enables assigning variants to the most appropriate therapeutic pathway. However, for a number of epilepsy-associated genes and variants, pathogenic mechanisms remain incompletely understood, and the functional consequences of a substantial proportion of identified variants have yet to be definitively established (18).

The clinical implementation of high-throughput sequencing and functional genomics has enabled more accurate molecular stratification of genetic epilepsies and created new opportunities to design precision therapies that target disease-causing mechanisms. By modulating pathogenic genes or correcting disease-associated sequences at the nucleic acid level, gene therapy offers a promising strategy for addressing the diagnostic and therapeutic challenges posed by refractory epilepsy. In recent years, numerous therapeutic platforms, including antisense oligonucleotides (ASOs), clustered regularly interspaced short palindromic repeats (CRISPR)-based transcriptional activation systems, adeno-associated virus (AAV)-mediated gene delivery, mRNA replacement therapy and RNA editing technologies, have advanced considerably. In multiple genetic epilepsy animal models, these approaches have substantially reduced seizure frequency, improved neurological function and extended survival, and some have progressed to early-phase clinical trials (19-21). Collectively, these advances indicate that epilepsy treatment is moving beyond symptomatic management towards mechanism-based precision medicine.

Despite this progress, gene therapy approaches remain heterogeneous in their mechanisms of action, mutation suitability and translational readiness. From a molecular pathophysiological perspective, therapeutic strategies for LOF variants generally focus on restoring gene activity through endogenous gene activation or exogenous gene replacement, whereas GOF variants typically require allele-specific suppression using approaches such as ASOs, RNA interference or CRISPR interference (CRISPRi) (22-24). Matching diverse therapeutic platforms to the complex, often variant-specific functional consequences of pathogenic mutations has emerged as a major obstacle to clinical translation. Although gene-correction technologies, particularly DNA and RNA editing, theoretically offer the possibility of durable or even one-time therapeutic interventions, their clinical application remains limited by challenges including insufficient delivery efficiency, the risk of off-target effects and uncertainty regarding long-term biosafety (25).

The present review adopts mutation-specific functional consequences (LOF vs. GOF) as the principal framework for therapeutic decision-making and systematically examines gene therapy strategies for genetic epilepsy. The intervention logic for LOF and GOF variants is discussed first, followed by

a comparative evaluation of five major therapeutic platforms: ASOs, regulatory AAV-based systems, CRISPR activation (CRISPRa) and CRISPRi, DNA editing and RNA editing. These approaches are assessed for translational maturity, delivery feasibility, therapeutic durability and safety considerations in paediatric populations. Finally, the major barriers to clinical translation are summarised, and a milestone-based translational evaluation framework is proposed to guide the selection and development of gene therapy strategies for genetic epilepsy.

2. Therapeutic strategies oriented to LOF mutations: Expression enhancement

LOF-associated genetic epilepsies are primarily caused by haploinsufficiency or dominant-negative variants that reduce functional protein expression. Consequently, the principal therapeutic objective is to restore gene dosage or enhance endogenous gene expression to levels sufficient for normal neuronal function. The present section focuses on three major therapeutic approaches designed to achieve this goal: Gene supplementation, regulatory AAV-mediated enhancement of endogenous expression and CRISPRa-based endogenous gene upregulation. Each strategy compensates for reduced gene function through distinct molecular mechanisms and differs in applicability, durability, delivery requirements and clinical development stage.

Gene supplementation. Gene supplementation (gene addition) involves delivering functional cDNA to target cells using viral or non-viral vectors to compensate for LOF caused by pathogenic variants. This approach is particularly suitable for disorders in which LOF mutations result in partial or complete loss of protein activity.

DS is a prototypical indication for gene supplementation therapy (17). The disorder is predominantly caused by heterozygous LOF variants in *SCN1A*, leading to $Na_v1.1$ haploinsufficiency in inhibitory interneurons and disrupting the excitatory-inhibitory balance within neural networks (26). However, the *SCN1A* coding sequence is ~6 kb, exceeding the packaging capacity of a single AAV vector (~4.7 kb). As a result, *SCN1A* supplementation requires split-gene approaches using dual-AAV systems that reconstitute the full-length transgene after delivery. Mich *et al* (27) showed that a dual-AAV vector platform could mediate SCN1A recombination in inhibitory interneurons in a mouse model of DS, restoring $Na_v1.1$ expression to ~60% of wild-type levels and reducing seizure frequency by ~80%. These findings provide proof-of-concept evidence supporting the feasibility of gene supplementation for LOF epilepsies involving large genes.

Despite its therapeutic potential, gene supplementation faces several important challenges. First, vector cargo limitations remain a major constraint. For large genes such as *SCN1A* and methyl-CpG binding protein 2 (*MECP2)*, the recombination efficiency, manufacturing complexity and long-term safety of dual- or triple-AAV systems require further optimisation. Second, uncontrolled transgene expression may result in supraphysiological protein levels, potentially causing neurotoxicity or functional disturbances. Consequently, incorporating cell-type-specific promoters, such as glutamic acid decarboxylase 67 (*GAD67*)-based regulatory elements, is a key strategy for improving therapeutic precision. Third, although AAV-mediated transgene expression can persist for months to years, long-term administration may elicit neutralising antibodies or cellular immune responses that could affect efficacy and safety.

Regulatory AAV-mediated enhancement of endogenous expression. To overcome the cargo limitations and potential overexpression risks associated with conventional gene supplementation, regulatory AAV-based approaches have emerged as an alternative strategy. Rather than delivering a full-length coding sequence, these systems introduce engineered regulatory elements or transcriptional modulators that enhance endogenous target gene expression. This approach restores protein levels while maintaining the native genomic context and physiological regulatory mechanisms.

ETX101, developed by Encoded Therapeutics, Inc., represents a leading example of this strategy (21,28). ETX101 is a recombinant AAV9-based regulatory gene therapy designed to selectively increase endogenous *SCN1A* expression in inhibitory interneurons using cell-type-specific regulatory elements. Preclinical studies demonstrated that ETX101 increased *SCN1A* mRNA expression in GABAergic interneurons and elevated $Na_v1.1$ protein levels in the brains of DS mouse models. Treatment reduced the mean daily frequency of generalised seizures by 68% and improved seizure severity as assessed by video-electroencephalogram recordings (21). Studies in non-human primates further demonstrated widespread central nervous system distribution without evident immunotoxicity or dose-limiting adverse effects. ETX101 has subsequently progressed to a Phase I clinical trial (NCT05419492), in which a single intracerebroventricular administration is being evaluated for safety and preliminary efficacy in paediatric patients with DS (21,28). This programme represents the first systematic clinical translation of a regulatory AAV platform for LOF-associated epilepsy.

Regulatory AAV-based therapies offer numerous notable advantages over conventional gene supplementation approaches. First, as they do not require delivery of a full-length coding sequence, they largely circumvent vector cargo limitations and are therefore particularly attractive for disorders involving large genes that exceed the packaging capacity of standard AAV vectors. Second, by enhancing transcription of the endogenous gene rather than introducing an exogenous transgene, they preserve the native regulatory environment, allowing gene expression to remain under the control of endogenous promoters and enhancers and thereby reducing the risk of supraphysiological protein expression. Third, cell-type specificity can be achieved through selective promoters and regulatory elements, restricting therapeutic effects to disease-relevant neuronal populations while minimising off-target expression. Such targeted regulation may improve both efficacy and safety, particularly in neurological disorders in which the pathogenic mechanism is confined to specific cell types, such as inhibitory interneurons in DS.

CRISPRa-mediated endogenous gene activation. CRISPRa is a CRISPR-based transcriptional activation technology in which nuclease-deactivated Cas9 (dCas9) is fused to transcriptional

activator domains, such as VP64, VPR or the synergistic activation mediator (SAM) complex, and directed by guide RNAs (gRNAs) to promoter or enhancer regions of target genes, thereby increasing endogenous transcription (29-31). Unlike gene supplementation or regulatory AAV-based approaches, CRISPRa does not introduce an exogenous protein-coding sequence. Instead, it enhances endogenous gene expression through transcriptional and epigenetic regulation, preserving the native genomic context and potentially providing a more physiologically controlled therapeutic effect.

In a mouse model of DS, authors delivered a dCas9-VP64 complex together with a specific gRNA using an AAV vector to activate the endogenous *Scn1a* locus. The objective was to restore $Na_v1.1$ expression from haploinsufficient levels (~50% of wild-type expression) to near-physiological levels, rather than inducing overexpression. CRISPRa-mediated activation restored $Na_v1.1$ expression to near-wild-type levels, notably reduced seizure frequency and improved motor coordination in treated animals (31). Fig. 1 illustrates the mechanism and workflow of CRISPRa-mediated targeted *SCN1A* activation in epilepsy.

Similarly, in a potassium voltage-gated channel subfamily A member 1 (*Kcna1*)-deficient mouse model, in which loss of the Kv1.1 potassium channel increases neuronal excitability, CRISPRa-mediated activation of *Kcna1* successfully restored potassium-channel function, reduced neuronal hyperexcitability, markedly decreased seizure frequency and improved cognitive performance (20). These findings provide proof-of-concept evidence supporting the use of CRISPRa for the treatment of LOF-associated epilepsies.

Tamura *et al* (32) extended this therapeutic paradigm to *SCN2A* haploinsufficiency. Using AAV-mediated CRISPRa delivery, the authors restored excitability in neocortical pyramidal neurons and protected *Scn2a*$^{+/-}$ mice against chemoconvulsant-induced seizures. Notably, therapeutic rescue remained achievable when treatment was administered during adolescence, suggesting a broader intervention window than previously assumed.

To enhance cellular specificity, investigators have incorporated inhibitory neuron-specific promoters, such as GAD67, to drive dCas9 expression selectively within disease-relevant neuronal populations. In a mouse model of DS, this strategy enabled selective activation of *SCN1A* in inhibitory interneurons, minimising potential adverse effects from non-specific activation throughout the brain (31).

CRISPRa offers several theoretical advantages. By activating endogenous transcription rather than introducing an exogenous coding sequence, it preserves the physiological regulatory architecture of the target gene, including native promoters, enhancers and untranslated regions. Consequently, CRISPRa may reduce the risk of supraphysiological protein expression that can occur with conventional cDNA-based gene supplementation. Furthermore, because CRISPRa targets only regulatory elements rather than full-length coding sequences, it may be particularly advantageous for large genes that exceed the packaging capacity of standard AAV vectors.

However, CRISPRa also presents several important challenges. Although it avoids exogenous gene replacement, the approach still depends heavily on efficient delivery of large genetic constructs. dCas9-activator fusion proteins are ~4.2 kb, and including gRNA expression cassettes often requires dual-AAV or alternative delivery systems, such as lipid nanoparticles (LNPs). In addition, therapeutic efficacy depends on sustained expression of both dCas9 and gRNAs, raising questions regarding long-term vector persistence, immunogenicity and durability of gene activation. Target engagement may also vary with chromatin accessibility and local epigenetic context, potentially affecting the consistency of therapeutic responses.

By contrast, conventional gene supplementation delivers the therapeutic cDNA directly and therefore requires maintenance only of the transgene itself rather than a transcriptional activation machinery. However, this approach remains constrained by vector cargo limitations (33) and the risk of promoter-driven overexpression. Consequently, the choice between CRISPRa and gene supplementation balances physiological regulation and technological complexity. CRISPRa offers endogenous gene control, cell-type-specific activation and suitability for large genes, whereas gene supplementation benefits from a more established translational pathway and a comparatively simpler therapeutic design. Although CRISPRa remains preclinical, accumulating evidence suggests it is a promising platform for treating LOF-associated genetic epilepsies. The principal advantages and challenges of CRISPRa are summarised in Table I (19,33,34).

Overall, CRISPRa provides a precise therapeutic strategy for LOF epilepsies without exogenous gene replacement. However, clinical translation still requires resolving key issues such as delivery efficiency, long-term safety and cell-type specificity.

3. Therapeutic strategies oriented towards GOF mutations: Gene silencing

GOF epilepsies are caused by increased channel activity, reduced depolarization thresholds or abnormal persistent currents, and the therapeutic goal is to selectively suppress expression of the pathogenic allele to block abnormal neuronal firing. The present section includes three main strategies: ASO-mediated silencing, RNA interference [small interfering (si)RNA] and CRISPRi.

ASO-mediated gene silencing. ASOs are currently the most translationally advanced gene-silencing platform for GOF epilepsies. ASOs are short, synthetic, single-stranded oligonucleotides (15-25 nucleotides) that bind to target RNA through Watson-Crick base pairing (35). Their mechanism of action depends on the chemical modifications and design of the ASO and can be broadly categorised into two major classes (36).

Gapmer ASOs [ribonuclease H (RNase H)-mediated degradation]. Gapmer ASOs contain a central DNA 'gap' flanked by chemically modified (2'-O-methoxyethyl or locked nucleic acid) 'wings'. Upon binding to target mRNA, the DNA-RNA hybrid duplex recruits endogenous RNase H, which specifically recognises and cleaves the RNA strand of the hybrid, degrading the target mRNA and suppressing pathological gene expression. This mechanism suits GOF mutations because it reduces mutant transcript abundance. Allele selectivity can be achieved by designing gapmer ASOs

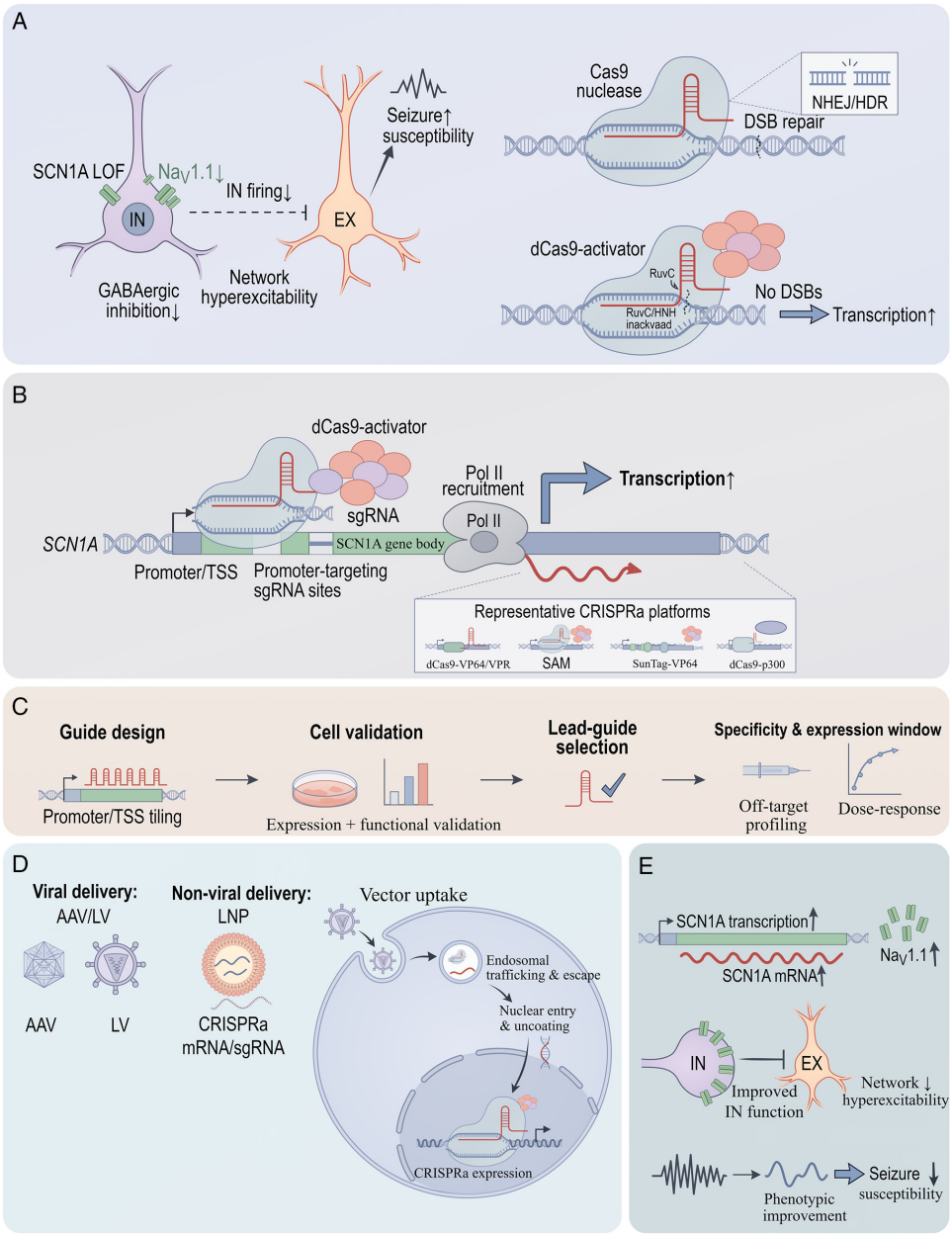


Figure 1. Mechanisms and workflow of CRISPR activation for targeted *SCN1A* upregulation in epilepsy. This figure illustrates the molecular mechanisms, guide design workflow, delivery strategies and functional outcomes of CRISPRa for therapeutic upregulation of *SCN1A* in genetic epilepsy. (A) Disease rationale and conceptual framework. LOF or haploinsufficiency of *SCN1A* reduces $Na_V1.1$ expression in GABAergic inhibitory interneurons, leading to impaired inhibitory firing, network hyperexcitability and seizures. By contrast to wild-type Cas9, which generates DSBs repaired by NHEJ or HDR, dCas9 retains DNA-targeting capacity but lacks endonuclease activity, thereby enabling programmable transcriptional activation without DNA cleavage. (B) Core mechanism of CRISPRa-mediated *SCN1A* activation. A dCas9-based activator complex is directed by an sgRNA to the *SCN1A* promoter, TSS region and where applicable validated CREs. Upon recruitment, the complex recruits transcriptional co-activators and RNA polymerase II to enhance endogenous *SCN1A* transcription. Representative CRISPRa architectures include direct fusion systems (such as dCas9-VP64), SAM, SunTag-based systems and epigenetic activators such as dCas9-p300, which act through transcriptional activation or local chromatin remodelling. (C) Guide design and optimization workflow. Candidate sgRNAs are designed across the promoter, TSS-proximal regulatory region and selected CREs, then validated in cells to identify lead guides with high on-target activity, appropriate activation strength and acceptable specificity. This step supports optimization of target engagement, expression amplitude and dose control before therapeutic deployment. (D) Delivery strategies and cell-type-targeted implementation. Therapeutic delivery may use viral (such as AAV) or non-viral platforms (such as LNPs). Given the large size of CRISPRa cargoes (~4.2 kb for dCas9-activator fusions plus gRNA expression cassettes), dual-AAV or split-delivery approaches represent practical strategies, whereas compact single-vector systems remain under development. After cellular uptake, endosomal escape and nuclear targeting, CRISPRa components assemble at the endogenous *SCN1A* locus. Interneuron-targeted delivery or interneuron-specific promoter-driven expression is emphasized to restore $Na_V1.1$ preferentially in inhibitory neuronal populations, thereby minimizing the risk of nonspecific activation in excitatory neurons. (E) Molecular, cellular and functional consequences. Enhanced *SCN1A* transcription increases *SCN1A* mRNA and $Na_V1.1$ protein expression, restoring sodium channel function in inhibitory interneurons. This promotes recovery of interneuron excitability, improves excitatory-inhibitory balance and provides a mechanistic basis for seizure reduction and circuit-level rescue. The therapeutic goal is physiologic restoration of endogenous *SCN1A* expression, typically to near-normal levels (80-100% of wild-type), rather than uncontrolled overexpression. Solid arrows indicate activation, causal flow, or workflow progression; dashed arrows indicate validation, optional or emerging steps; T-shaped lines indicate inhibition. AAV, adeno-associated virus; CRE, cis-regulatory element; CRISPR, clustered regularly interspaced short palindromic repeats; CRISPRa, CRISPR activation; DSB, double-strand break; dCas9, nuclease-deactivated Cas9; HDR, homology-directed repair; LOF, loss-of-function; LNP, lipid nanoparticle; $Na_V1.1$, voltage-gated sodium channel $Na_V1.1$; NHEJ, non-homologous end joining; SAM, synergistic activation mediator; *SCN1A*, sodium voltage-gated channel alpha subunit 1; sgRNA, single guide RNA; TSS, transcription start site.

Table I. Advantages and challenges of clustered regularly interspaced short palindromic repeats activation.

Advantages	Challenges
Non-integrating; does not introduce exogenous cDNA	Large delivery payload (deactivated Cas9 is ~4.2 kb; together with activators and gRNA, delivery often requires dual adeno-associated virus or lipid nanoparticle systems) (33,34)
Expression levels are tuneable (through optimization of gRNA design and promoter selection)	gRNA target-site design depends on chromatin accessibility and requires cross-species validation (34)
Theoretically reversible (through cessation of expression or use of inducible systems)	Long-term expression may interfere with other genenetworks, and safety still requires systematic evaluation (19)
Preserves the physiological regulatory architecture of endogenous genes	Still in the preclinical stage and lacks human data

gRNA, guide RNA.

that specifically target single-nucleotide polymorphisms or mutation-specific sequences, thereby sparing the wild-type allele. Examples of gapmer ASOs in epilepsy include the *SCN8A*-targeting ASO developed by Lenk *et al* (37), which reduced mutant *SCN8A* mRNA by ~50% while preserving wild-type expression, and the potassium sodium-activated channel subfamily T member 1 (*KCNT1*)-targeting ASO developed by Burbano *et al* (38), which selectively degraded mutant *KCNT1* transcripts.

Splice-switching ASOs. By contrast, splice-switching ASOs do not recruit RNase H. Instead, they bind to pre-mRNA at splice sites, exonic splicing enhancers or intronic splicing silencers, physically blocking access by splicing factors and thereby modulating alternative splicing. This mechanism can skip mutated exons, restore correct reading frames or increase the production of functional transcripts. Splice-switching ASOs are particularly valuable for LOF mutations caused by aberrant splicing. A prominent example in epilepsy is STK-001 (developed by Stoke Therapeutics, Inc.), which targets a negative splicing enhancer region in *SCN1A* pre-mRNA, increasing functional *SCN1A* mRNA and restoring $Na_v1.1$ protein expression in DS (39). As splice-switching ASOs do not degrade RNA, they preserve the transcript pool and are generally associated with lower toxicity than gapmers (40).

Both ASO classes offer strong allele selectivity when designed against mutation-specific sequences, controllable dosing and good reversibility, making them attractive for paediatric applications that may require dose titration and treatment adjustment.

SCN8A-associated encephalopathy. GOF mutations in *SCN8A* enhance $Na_v1.6$ channel function, triggering spontaneous neuronal firing and persistent seizures. In an *SCN8A-R1872W* knock-in mouse model, Lenk *et al* (37) demonstrated that intracerebroventricular administration of a non-allele-specific ASO reduced mutant mRNA levels by ~50%, decreased $Na_v1.6$ expression by ~50%, reduced seizure frequency by >70% and extended median survival from 15 days to 7 weeks. The ASO preserved wild-type allele expression, avoiding potential adverse effects such as motor dysfunction. Follow-up studies further confirmed that long-term ASO treatment can durably prevent seizures and prolong survival (36,41).

KCNT1-associated encephalopathy. Missense *KCNT1* mutations (such as p.Arg428Gln) abnormally enhance the function of the sodium-activated potassium channel (also known as the Slack channel), causing severe early-onset developmental and epileptic encephalopathy. Burbano *et al* (38) designed an allele-selective ASO targeting this mutation. Through RNase H-mediated degradation of mutant mRNA, the ASO markedly reduced expression of the abnormal channel protein. In a mouse model of *KCNT1* encephalopathy, ASO treatment reduced seizure frequency by >70% without affecting wild-type allele expression (38). Separately, *KCNT1*-lowering ASOs prolonged survival in *SCN1A* and *SCN8A* mutant mice (42). Andreone *et al* (43) further validated the efficacy of divalent siRNA in a preclinical model of *KCNT1* genetic epilepsy, demonstrating durable seizure suppression.

SCN2A-associated encephalopathy. ASO-mediated silencing is also applicable to GOF mutations in *SCN2A*. Li *et al* (44) validated the therapeutic efficacy of ASOs in a mouse model of *SCN2A* GOF mutations and observed marked reductions in seizure frequency, along with prolonged survival. In a landmark clinical study, Kim-McManus *et al* (45) reported two N-of-1 trials using allele-selective ASOs for *SCN2A*-associated developmental and epileptic encephalopathy, in which one patient experienced a 90% reduction in seizure frequency and walked independently for the first time at 15 years of age, without serious adverse events. Fig. 2 illustrates the mechanisms of ASO therapy for *SCN2A*- and *SCN8A*-associated epilepsies.

Collectively, these studies indicate that ASO-mediated silencing is an effective, controllable and clinically advanced therapeutic strategy for GOF channelopathies. At present, ASO therapy targeting *SCN8A* has entered a key transition phase from preclinical research to clinical translation.

siRNA and LNP delivery. RNA interference is a gene-silencing mechanism mediated by siRNAs. After complementary binding to target mRNA, siRNAs recruit and activate the RNA-induced silencing complex, which promotes cleavage and degradation of the target mRNA, thereby rapidly and efficiently suppressing gene expression (46).

In recent years, LNP-based siRNA delivery systems have shown substantial improvements in *in vivo* stability

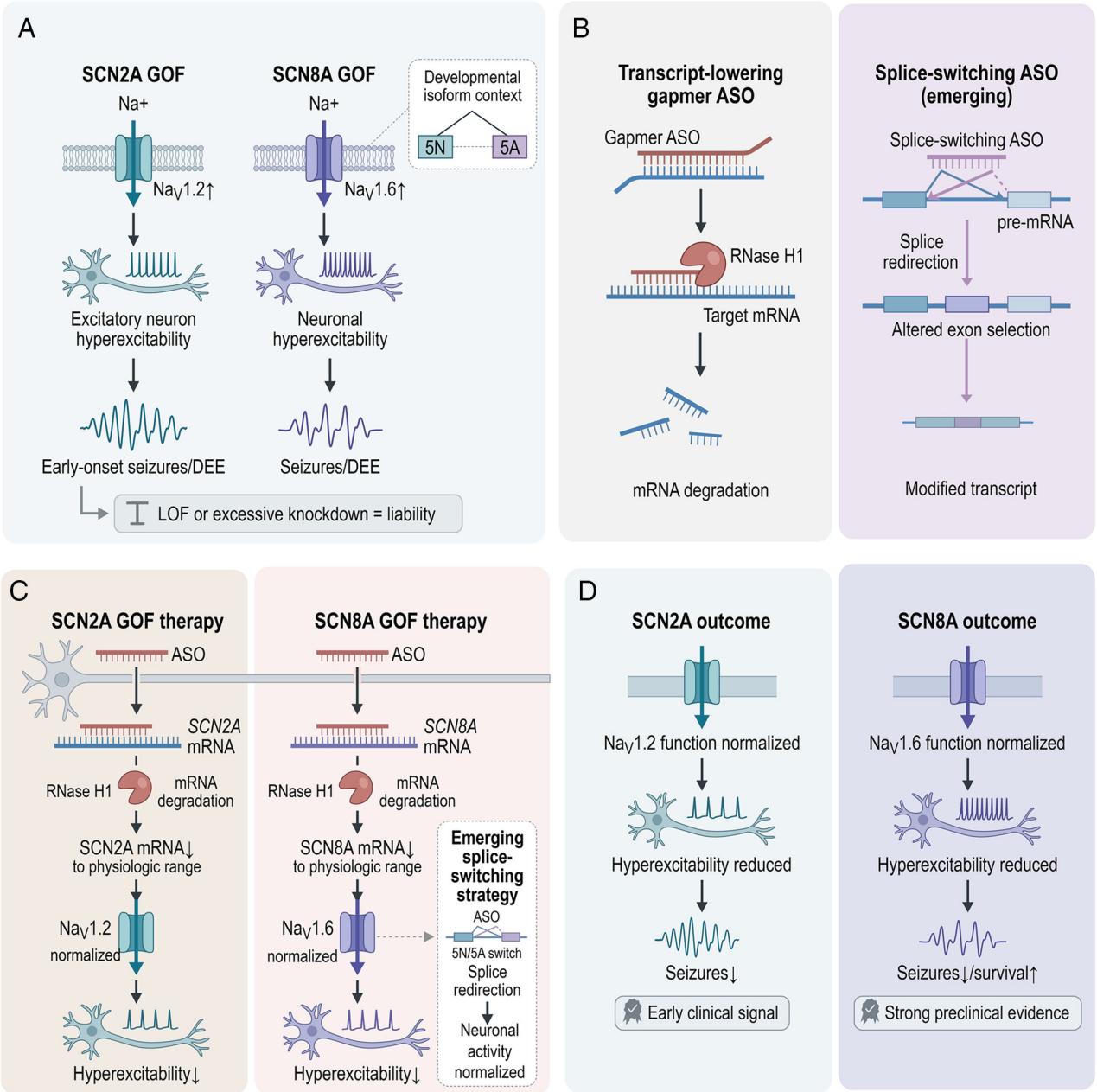


Figure 2. Mechanisms of antisense oligonucleotide therapy for *SCN2A*- and *SCN8A*-associated epilepsies. This figure illustrates the molecular mechanisms, gene-specific applications and therapeutic outcomes of ASO-based strategies for GOF epilepsies caused by mutations in *SCN2A* and *SCN8A*. (A) Variant stratification and disease rationale. The figure focuses on GOF forms of *SCN2A*- and *SCN8A*-associated epilepsies, in which increased $Na_V1.2$ or $Na_V1.6$ activity drives neuronal hyperexcitability and seizures. A small developmental isoform context is included to indicate that alternative splicing contributes to channel biology and therapeutic opportunities; however, LOF states are presented only as a cautionary context rather than a primary treatment indication. This distinction is important because transcript-lowering strategies are primarily being developed for GOF-associated disease, whereas excessive suppression of channel expression may itself be deleterious in LOF contexts. (B) Core ASO mechanisms. The principal mechanism shown is gapmer ASO-mediated transcript lowering, in which the ASO binds target mRNA, forms an ASO:mRNA hybrid, recruits RNase H1 and promotes degradation of the pathogenic transcript. A secondary, emerging mechanism is splice switching, in which an ASO binds pre-mRNA and redirects splice-site usage or isoform selection to favour production of a more functional transcript. Other ASO mechanisms, such as translational blockade and microRNA modulation, are not emphasised, as they are not the predominant evidence-supported therapeutic approaches for these sodium channel epilepsies. (C) Gene-specific therapeutic mechanisms. For *SCN2A* GOF epilepsies, a transcript-lowering ASO reduces *SCN2A* mRNA through RNase H1-mediated degradation, thereby reducing excess $Na_V1.2$ activity towards a physiological range and decreasing neuronal hyperexcitability. For *SCN8A* GOF epilepsies, a similar transcript-lowering strategy reduces *SCN8A* mRNA and pathogenic $Na_V1.6$ activity. An additional, smaller branch illustrates an emerging *SCN8A* splice-switching ASO strategy, which redirects pre-mRNA processing to normalise functionally relevant isoforms. The therapeutic goal in both settings is controlled normalisation of channel activity rather than indiscriminate or complete silencing, which could result in haploinsufficiency or other adverse effects. (D) Functional rescue and therapeutic outcomes. ASO treatment is intended to normalise channel activity, reduce pathological neuronal firing and improve seizure outcomes. The evidence tier differs between the two genes: *SCN2A* has early clinical proof of concept for transcript-lowering therapy in early-onset GOF developmental and epileptic encephalopathy, with a recent case report demonstrating ASO treatment in a preterm infant (72); *SCN8A* currently has strong preclinical evidence supporting transcript lowering, including benefit after seizure onset and with repeated dosing in animal models. A cautionary note highlights that dose titration and genotype-specific use are required to avoid over-suppression, as excessive channel reduction may compromise normal neuronal function. Solid arrows indicate causal flow or therapeutic progression; dashed arrows indicate emerging or lower-evidence branches. ASO, antisense oligonucleotide; DEE, developmental and epileptic encephalopathy; GOF, gain-of-function; LOF, loss-of-function; mRNA, messenger RNA; pre-mRNA, precursor messenger RNA; RNase H1, ribonuclease H1; *SCN2A*, sodium voltage-gated channel alpha subunit 2; *SCN8A*, sodium voltage-gated channel alpha subunit 8.

and tissue targeting. Angiopep-2-modified LNPs (Ang-C2 LNPs), developed by Tong *et al* (47), can effectively cross the blood-brain barrier (BBB), deliver siRNA efficiently to brain tissue and maintain target-gene suppression for ~15 days (47,48). Andreone *et al* (43) further validated the efficacy of divalent siRNA in a preclinical model of *KCNT1*-associated genetic epilepsy and demonstrated durable seizure suppression. However, compared with ASOs, siRNA delivery to the central nervous system still faces several challenges.

Shorter half-life. The *in vivo* half-life of siRNAs is typically 1-2 days, considerably shorter than the 2-4 weeks reported for ASOs, necessitating more frequent dosing or more sophisticated delivery systems.

Delivery efficiency. siRNAs depend on delivery vehicles such as LNPs to cross the BBB, and the cell-type specificity and long-term safety of these carriers require further optimisation.

Limited clinical evidence. To date, no published study has directly demonstrated improvement of epileptic phenotypes in *SCN2A* or *SCN8A* animal models using siRNA.

CRISPRi. CRISPRi is an epigenetic repression technology that does not involve DNA double-strand breaks (DSBs). By fusing nuclease-dCas9 to a transcriptional repressor, such as the Krüppel associated box (KRAB) domain, and directing the complex to a gene promoter using a gRNA, CRISPRi promotes the deposition of repressive histone marks, including H3K9me3, enabling reversible, programmable gene silencing (29). In neuronal systems, AAV-mediated CRISPRi delivery has been shown to suppress target-gene expression effectively. Duke *et al* (49) optimised a neuron-specific CRISPRi system and achieved efficient repression of endogenous genes in primary cortical neurons.

Compared with ASOs and siRNA, CRISPRi offers more durable gene repression as epigenetic modifications are stable and can simultaneously regulate multiple genes using distinct gRNAs. However, several challenges continue to limit its clinical translation: i) Large delivery payload, the dCas9-KRAB fusion protein is ~4.5 kb and, together with gRNA expression elements, often requires delivery via dual AAV vectors or LNP-based systems; cell-type specificity, selective repression of target cell populations requires cell-type-specific promoters; and iii) long-term safety, the durability of epigenetic modifications and their potential off-target effects require further systematic evaluation (34,50,51). At present, CRISPRi in epilepsy remains at the preclinical optimisation stage.

4. Precision editing strategies: DNA editing and RNA editing

Precision editing technologies aim to correct pathogenic mutations at their source and therefore have the theoretical potential to provide one-time curative treatment. Depending on the molecular substrate targeted, these approaches can be divided into DNA editing and RNA editing. These two strategies differ substantially in their reversibility, durability, safety profiles and translational maturity and are therefore discussed separately in this section.

DNA editing. DNA editing corrects mutations by permanently modifying genomic sequences. The major platforms include CRISPR/Cas9, base editing and prime editing.

CRISPR/Cas9. Under the guidance of a single-gRNA (sgRNA), the CRISPR/Cas9 system recognises a target site and induces a DNA DSB, after which non-homologous end joining (NHEJ) facilitates gene knockout, whereas homology-directed repair (HDR) using a donor template enables the correction of point mutations (49,52). However, HDR is cell cycle-dependent and inefficient in terminally differentiated neurons, limiting its direct application in adult neurological disorders. Consequently, in epilepsy, CRISPR/Cas9 has primarily been employed in knockout-based strategies targeting GOF mutations.

Base editing. Base editing overcomes the conventional reliance of conventional gene editing on DNA DSB. By fusing a Cas9 nickase to a deaminase, it directly mediates targeted single-base substitutions without cleaving both DNA strands. The most widely used base editors include cytosine base editors, which mediate C-G to T-A conversions, and adenine base editors (ABEs), which mediate A-T to G-C conversions (53).

In *SCN8A* GOF epilepsy models, base editing has demonstrated promising therapeutic efficacy. Yu *et al* (13) used base editing to target a dominant mutant *SCN8A* allele and successfully corrected the pathogenic variant in a mouse model, thereby protecting the animals from seizures and premature mortality. Cao *et al* (54) subsequently applied prime editing to correct a pathogenic mutation in a mouse model of genetic epilepsy, ameliorating neuronal hyperexcitability and seizure activity. In a separate study, Reever *et al* (55) employed adenine base editing to correct the recurrent *SCN8A* R1872W mutation, achieving ~30% conversion of mutant transcripts to the wild-type sequence. This level of correction was sufficient to notably prolong survival and completely prevent seizures in 7/11 treated mice (55).

Prime editing. Prime editing further expands the spectrum of editable genetic variants. By combining a Cas9 nickase with a reverse transcriptase and using a prime editing gRNA (pegRNA), prime editing enables diverse base substitutions, as well as short insertions and deletions, without donor DNA or the induction of DNA DSBs (56). Unlike base editing, which is largely restricted to transition mutations, prime editing can correct a broader range of pathogenic variants, including frameshift mutations and short deletions.

Robertson *et al* (57) optimised a prime-editing platform in primary mouse neurons and established a methodological framework for neuronal genome editing relevant to epilepsy research. The study developed standardised protocols for pegRNA and prime editor 3b (PE3b) nicking guide RNA design, plasmid construction, primary neuronal nucleofection and quantitative next-generation sequencing analysis. Although the study did not directly evaluate anti-epileptic efficacy, it showed that prime editing can precisely rewrite pathogenic neuronal variants while preserving the endogenous regulatory environment. These findings provide important technical guidance for functional modelling and future therapeutic editing studies involving epilepsy-associated genes such as *SCN1A, SCN2A, SCN8A* and *GRIN2A* (57).

Cerna-Chavez *et al* (58) subsequently demonstrated efficient prime editing in human iPSCs (hiPSCs), enabling efficient site-specific modification of point mutations, small insertions and small deletions and facilitating the

Table II. Advantages and challenges of RNA editing.

Advantages	Challenges
Reversibility: Editing effects are not permanent and can be adjusted or terminated	Limited durability: As RNA has a short half-life, repeated dosing may be required
Does not alter the genome, thereby avoiding the risk of genomic integration	Delivery efficiency: Adeno-associated virus- orlipid nanoparticle-based delivery systems still require optimization
Independent of cell division and therefore suitable for terminally differentiated neurons	Off-target editing: Transcriptome-wide A-to-I off-target effects require systematic evaluation
Dose is tuneable and thus suitable for paediatric application	Immunogenicity of the editing enzyme: Exogenous adenosine deaminase acting on RNA may trigger immune responses

generation of isogenic disease models for multiple monogenic disorders. Although the study did not specifically examine epilepsy-associated genes, it suggests that prime editing is technically feasible for precisely introducing or correcting pathogenic variants in patient-derived hiPSCs carrying mutations in genes such as *SCN1A*, *SCN8A* and *KCNQ2*. Together with subsequent work from the Robertson group (56), which used in utero prime editing to introduce an ultra-rare *GRIN2A* epilepsy-associated variant and induce spontaneous seizures, these findings indicate that prime editing in epilepsy is extending beyond *in vitro* disease modelling toward personalised validation of neurodevelopmental and seizure-associated phenotypes (59). In addition, Cao *et al* (54) applied PE3-mediated prime editing in *Kcnq2*^{R207W} mice with genetic epilepsy to directly correct the pathogenic variant. Site-specific repair reduced neuronal hyperexcitability and seizure frequency, providing one of the few preclinical demonstrations of prime editing as a therapeutic intervention for genetic epilepsy (54).

Core challenges of DNA editing. Despite their transformative potential, DNA-editing technologies face substantial challenges that temper expectations for near-term clinical translation. The irreversible nature of genomic modification requires markedly high editing specificity and safety, particularly when applied to the developing brain. Delivery efficiency remains a major obstacle, as base-editing and prime-editing platforms generally exceed the packaging capacity of a single AAV vector. In addition, off-target editing at both the genomic and transcriptomic levels, although reduced compared with conventional CRISPR/Cas9 systems, has not been completely eliminated. The long-term consequences of permanent genomic modification in the paediatric brain remain unknown, while the therapeutic window for intervention in numerous early-onset epilepsies are limited. Collectively, these considerations indicate that, although DNA editing offers the prospect of curative treatment, its clinical implementation should proceed cautiously and be restricted to situations in which the anticipated benefits outweigh the potential risks (60).

RNA editing. RNA editing restores protein function by modifying bases within mutant mRNA transcripts without permanently altering genomic DNA. Compared with DNA editing, RNA editing offers greater reversibility, a lower risk of off-target effects and independence from cell cycle activity, making it particularly well suited to non-dividing neurons within the central nervous system (61).

Adenosine deaminase acting on RNA (ADAR)-mediated A-to-I editing. The most prevalent form of RNA editing is adenosine deamination mediated by the ADAR family of enzymes. ADAR converts A to I, which is interpreted as G during translation, thereby producing the functional equivalent of an A-T to G-C base substitution (61). Artificial RNA-editing systems typically achieve site-specific editing by fusing the catalytic domain of ADAR to a guide RNA-directed targeting platform or by using engineered oligonucleotides to recruit endogenous ADAR to the target transcript. i) Recruiting Endogenous ADAR to Specific Transcripts for Oligonucleotide-mediated RNA Editing (RESTORE) platform. Merkle *et al* (62) developed the RESTORE system, which employs engineered oligonucleotides to recruit endogenous ADAR and facilitate efficient RNA repair. As the platform delivers no exogenous editing enzyme, it is associated with low immunogenicity.

ii) Leveraging Endogenous ADAR for Programmable Editing of RNA 2.0. Yi *et al* (63) utilised engineered circular RNA molecules to recruit endogenous ADAR, achieving durable and efficient RNA editing in non-human primates, with editing efficiencies >50% and persistence for ≥13 weeks (63,64). In a humanised mouse model of Hurler syndrome, the system successfully corrected a nonsense mutation, demonstrating therapeutic potential (63).

iii) Light-controlled RNA editing. Li *et al* (65) developed the photoactivatable RNA ABE system, which uses blue-light stimulation to regulate ADAR activity and enable spatiotemporal control of RNA editing. In mice, this approach reduced off-target editing to baseline levels.

Advantages and challenges of RNA editing. The principal advantages and challenges of RNA editing are summarised in Table II. The reversibility and genomic safety of RNA editing make it particularly appealing for paediatric applications. However, its transient nature, inherently associated to mRNA turnover, may require repeated administration to maintain therapeutic efficacy, creating challenges for long-term treatment in young children. Delivery efficiency also remains suboptimal, while transcriptome-wide off-target A-to-I editing continues to raise safety concerns that require comprehensive

evaluation. Additional challenges include the potential immunogenicity of exogenous ADAR enzymes and AAV-based delivery systems, as well as immune responses directed against edited transcripts.

Decision logic for DNA vs. RNA editing. RNA editing may be prioritised when paediatric safety, dose titration and reversibility are the principal considerations, particularly in patients undergoing active neurodevelopment. By contrast, DNA editing may be more appropriate when efficient delivery systems and long-term monitoring frameworks are available to support durable, irreversible therapeutic intervention and when the pathogenic variant has been identified and the anticipated clinical benefit is substantial. At present, RNA editing in epilepsy remains at the stage of preclinical validation. These selection considerations derive from methodological reviews and safety analyses (60,66), rather than direct head-to-head clinical comparisons of DNA and RNA editing in genetic epilepsy.

5. Translational research progress in representative epilepsy genes

The preceding sections systematically described the technical principles underlying three major approaches: LOF-oriented strategies, GOF-oriented strategies and precision editing. The present section uses specific genes as illustrative examples to highlight the current applications, key findings and translational progress of these strategies in genetic epilepsies. The discussion focuses primarily on *SCN1A* (DS), *SCN2A*/*SCN8A*-associated encephalopathies, *SCN3A* encephalopathy, *KCNQ2/3* mutation-associated epilepsy, mTOR pathway-associated epilepsy and synapse-associated genes, including GRIN2A, γ-aminobutyric acid receptor subunit γ-2 (*GABRG2*) and *STXBP1*.

SCN1A: DS-a paradigm of LOF epilepsy. DS is a prototypical LOF epilepsy. Heterozygous LOF mutations in SCN1A result in $Na_v1.1$ haploinsufficiency in inhibitory interneurons, disrupting the excitation-inhibition balance. As the *SCN1A* coding sequence is relatively large (~6 kb), conventional gene supplementation is limited by vector cargo capacity. Consequently, therapeutic development has shifted towards strategies that enhance endogenous *SCN1A* expression (17).

ASO therapy (STK-001). STK-001, developed by Stoke Therapeutics, Inc., is based on the Targeted Augmentation of Nuclear Gene Output platform and employs a sequence-specific ASO targeting a negative splicing enhancer within *SCN1A* pre-mRNA, thereby increasing the production of functional *SCN1A* mRNA (39). In $Scn1a^{+/-}$ mice, intrathecal administration of STK-001 increased brain *SCN1A* mRNA levels by ~50%, restored $Na_v1.1$ protein expression to near-normal levels, reduced spontaneous seizure frequency by ~70% and improved motor coordination and survival (39).

Phase I/II clinical trials (MONARCH, NCT04442295; SWALLOWTAIL, NCT04740476) have been completed. Publicly available data indicate that STK-001 is generally well tolerated, with no dose-limiting toxicities or serious adverse events reported (67). Some patients achieved a ≥50% reduction in seizure frequency, with trends toward improved cognitive and behavioural outcomes (68). These findings supported advancing STK-001 into a global pivotal phase III trial (EMPEROR, NCT06872125), making it one of the most clinically advanced gene-targeted therapies for DS (28,69,70).

Regulatory AAV (ETX101). ETX101, developed by Encoded Therapeutics, Inc., uses an engineered AAV9 vector to selectively upregulate endogenous *SCN1A* expression in inhibitory interneurons, thereby avoiding both full-length cDNA cargo constraints and the risk of exogenous overexpression (21,28). In DS mouse models, ETX101 markedly increased *SCN1A* expression, reduced seizure frequency and improved electrophysiological function. Studies in non-human primates demonstrated widespread central nervous system distribution without evident immunotoxicity (21). A Phase I clinical trial (NCT05419492) is currently evaluating the safety and preliminary efficacy of a single intrathecal administration in paediatric patients. As an early-stage clinical candidate, ETX101 represents a promising gene-regulatory approach for DS and is currently undergoing first-in-human evaluation (21,28).

CRISPRa. In DS mouse models, AAV-delivered dCas9-VP64 complexes activated the endogenous *Scn1a* gene in inhibitory interneurons, restoring $Na_v1.1$ expression to near-normal levels (80-100% of wild-type levels), markedly reducing seizure frequency and improving motor coordination (31). Human iPSC-derived GABAergic interneurons have instead been used to map *SCN1A* cis-regulatory elements relevant to future gene-activation strategies (71). Although these results are encouraging, this approach remains preclinical, with further optimisation and safety evaluation required before clinical translation. The major gene-targeted therapeutic strategies for DS and their translational status are compared in Table III.

SCN2A/SCN8A-associated encephalopathies: A paradigm of GOF epilepsy. SCN2A and *SCN8A* encode the $Na_v1.2$ and $Na_v1.6$ sodium channels, respectively. GOF mutations in these genes increase channel activity and lower the depolarisation threshold, resulting in early-onset epileptic encephalopathy, developmental delay and severe neurobehavioral impairment (9). In contrast to the LOF mechanism associated with *SCN1A*, these epilepsies are more amenable to gene-silencing strategies.

SCN8A ASO therapy. In an *SCN8A*-R1872W knock-in mouse model, Lenk *et al* (37) demonstrated that intracerebroventricular administration of a non-allele-specific ASO reduced mutant mRNA levels by ~50%, decreased $Na_v1.6$ expression by ~50%, reduced seizure frequency by >70%, and extended median survival from 15 days to 7 weeks (37). Expression of the wild-type allele was preserved, thereby avoiding adverse effects such as motor dysfunction. Hill *et al* (41) further demonstrated that long-term ASO therapy can provide sustained seizure prevention and prolong survival, supporting the progression of this approach from the preclinical stage towards clinical translation.

SCN2A ASO therapy. Li *et al* (44) validated the therapeutic efficacy of ASOs in a mouse model of *SCN2A* GOF mutations, demonstrating marked reductions in seizure

Table III. Summary comparison of Dravet syndrome strategies.

Strategy	Representative agent	Mechanism	Translational status	Key advantages	Main challenges
Antisense oligonucleotides upregulation	STK-001	Splicing modulation; increases functional mRNA	Phase III clinical	Reversible; dose-controllable; mature delivery	Requires repeated dosing
Regulatory adeno-associated virus	ETX101	Enhances endogenous gene transcription	Phase I clinical	Durable; single-dose administration; cell-type specific	Irreversible; immunogenicity
Clustered regularly interspaced short palindromic repeats activation	Preclinical	Deactivated Cas9-mediated transcriptional activation	Preclinical	Non-integrating; preserves physiological regulation	Complex delivery; unknown long-term safety

frequency and prolonged survival. Wagner *et al* (72) reported a case of a preterm infant with early-onset *SCN2A* encephalopathy treated with an ASO, demonstrating the feasibility of this approach in very-early-stage patients. Collectively, these findings support advancing *SCN2A*-targeted ASO therapy from preclinical studies toward early clinical exploration.

siRNA and CRISPRi. In *SCN8A*, AAV-delivered short hairpin RNA, a closely associated RNA interference modality to siRNA, reduced *SCN8A* expression, durably prevented seizures and prolonged survival throughout a 12-month observation period (73). Regarding CRISPRi, Duke *et al* (49) showed that a neuron-specific CRISPRi system efficiently repressed endogenous genes in primary cortical neurons. Collectively, these findings support the therapeutic potential of gene-silencing approaches targeting *SCN8A* and indicate that both RNA interference and CRISPRi strategies remain at the preclinical stage.

SCN3A encephalopathy-a complex phenotype combining LOF and GOF. *SCN3A* encodes the $Na_v1.3$ sodium channel. Pathogenic variants can result in either GOF or LOF effects and are frequently associated with cortical developmental abnormalities and early-onset epileptic encephalopathy (74). In iPSC-derived neuronal models, GOF variants, exemplified by p.I875T, recapitulate increased sodium currents and abnormal persistent neuronal firing (74).

At present, no ASO- or gene-editing strategy specifically targeting *SCN3A* has been formally reported. Clinical management therefore relies primarily on precision pharmacological approaches. Lacosamide and phenytoin inhibit $Na_v1.3$ activity and restore electrophysiological function (74). More recently, Qu *et al* (75) reported that a $Na_v1.3$-selective inhibitor restored abnormal electrical activity in iPSC-derived neurons.

Several factors have slowed the development of gene therapies for *SCN3A* compared with *SCN1A, SCN2A* and *SCN8A*. First, *SCN3A* was only firmly established as a cause of epileptic encephalopathy in 2018, whereas *SCN1A, SCN2A* and *SCN8A* had been recognised as major epilepsy genes >10 years earlier (14-16), providing substantially more time for preclinical and translational development. Second, *SCN3A*-associated disorders exhibit considerable phenotypic complexity, with manifestations that include cortical malformations such as polymicrogyria in addition to epilepsy. Consequently, effective therapies may need to address both electrophysiological dysfunction and structural neurodevelopmental abnormalities. Third, *SCN3A* variants display marked functional heterogeneity, producing GOF, LOF, or, in some cases, mixed effects. This complicates therapeutic selection, whereas *SCN1A* and *SCN8A* are associated predominantly with LOF and GOF mechanisms, respectively. Fourth, disease models have been comparatively limited, with animal models that faithfully reproduce both seizure phenotypes and cortical malformations emerging slowly, and iPSC-based platforms only recently becoming available for therapeutic evaluation (58). Finally, *SCN3A*-associated epilepsies are considerably rarer than *SCN1A*- or *SCN2A*-associated disorders (76,77), reducing commercial incentives and funding opportunities for therapeutic development. Collectively, these factors have positioned *SCN3A* as a later-stage candidate for gene-based intervention. However, recent advances in iPSC modelling and the development of $Na_v1.3$-selective small-molecule inhibitors provide a foundation for future therapeutic innovation. The pathogenic pathway underlying *SCN3A*-associated epileptic encephalopathy is illustrated in Fig. 3.

Future therapeutic strategies may draw on advances achieved in *SCN8A*- and *KCNT1*-associated disorders. For GOF variants, allele-specific ASOs could be developed to selectively suppress mutant transcripts. For LOF variants, RNA-editing approaches, such as ADAR-mediated A-to-I editing, may offer a reversible way to restore gene function. Overall, SCN3A-targeted gene therapy remains at an early exploratory stage, with research currently focused primarily on pharmacological intervention and with gene-based approaches yet to enter formal preclinical validation.

KCNQ2/3 mutation-associated epilepsy: Exploration of gene therapy for potassium-channel LOF. *KCNQ2* and *KCNQ3*

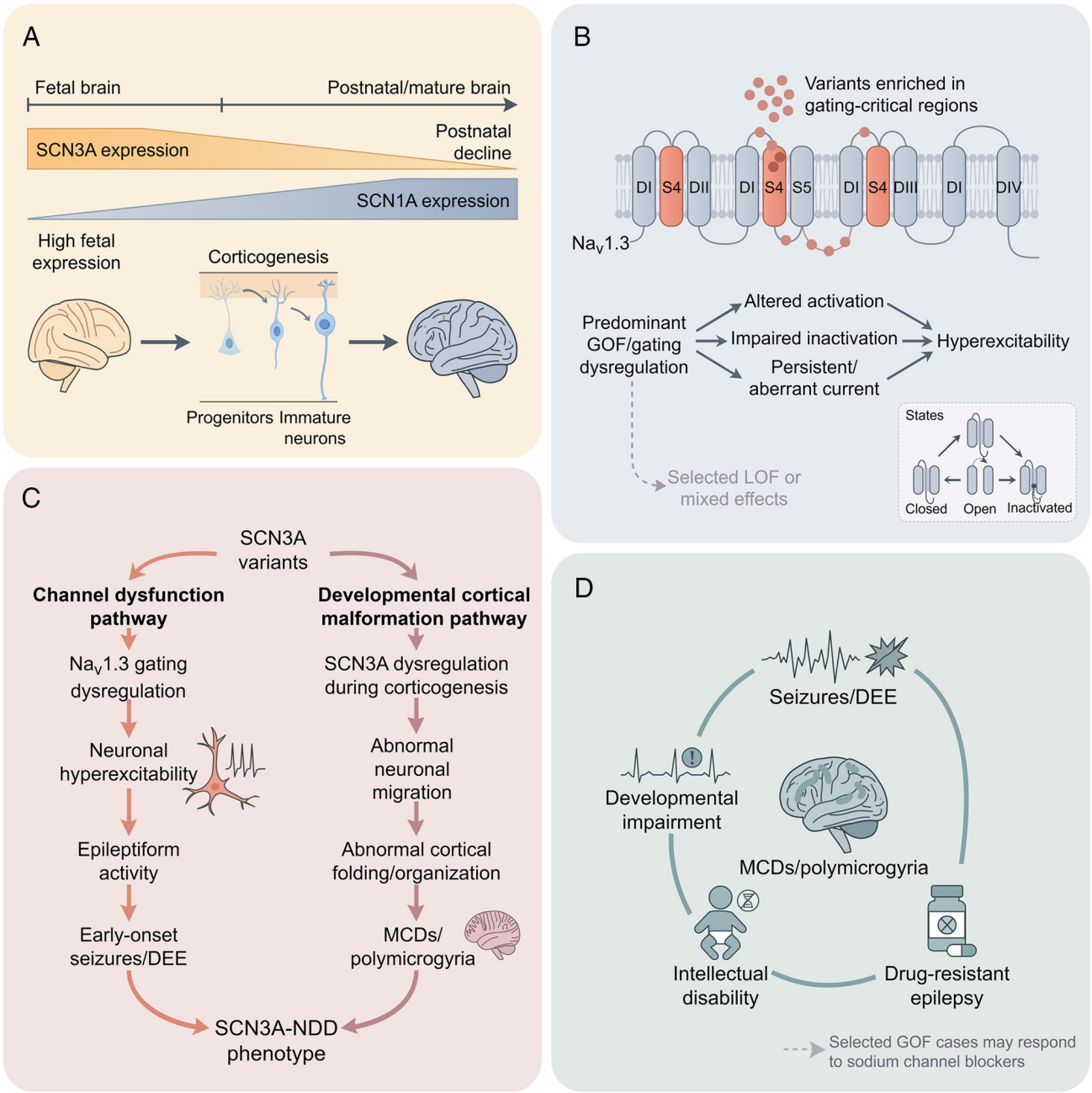


Figure 3. Pathogenic pathways of *SCN3A*-associated epileptic encephalopathy. This figure illustrates the developmental context, variant clustering, dual pathogenic mechanisms and integrated clinical spectrum of *SCN3A*-associated neurodevelopmental disorders, highlighting why this condition presents unique challenges for gene therapy development compared with *SCN1A*, *SCN2A* and *SCN8A*. (A) Developmental context of *SCN3A*. *SCN3A* encodes the voltage-gated sodium channel $Na_V1.3$, which is expressed during foetal and early brain development and declines postnatally. Its expression in cortical progenitors and immature neurons associates *SCN3A* dysfunction to early corticogenesis and helps explain why *SCN3A*-associated disease frequently combines epilepsy with MCDs, a phenotypic feature that distinguishes *SCN3A* from other sodium channel epilepsies and complicates gene therapy design. (B) Variant clustering and channel dysfunction. Pathogenic *SCN3A* variants are enriched in gating-critical transmembrane regions, including voltage-sensing and gating-coupling segments. Numerous disease-associated variants exert predominantly GOF effects, although variant-specific biophysical mechanisms are heterogeneous and may include altered activation, impaired inactivation or other gating abnormalities. A smaller subset exhibits LOF or mixed functional effects. This functional heterogeneity poses a major challenge for therapeutic strategy selection, as GOF mutations may be amenable to silencing approaches, whereas LOF mutations require enhanced expression, yet the same gene may harbour both mutation types. (C) Dual pathogenic pathways. One major branch arises from $Na_V1.3$ gating dysregulation, which increases neuronal excitability and promotes epileptiform activity, contributing to DEE. A second major branch reflects disruption of corticogenesis during foetal brain development, leading to abnormal neuronal migration, cortical organisational defects and MCDs, particularly polymicrogyria. Together, these parallel processes, electrophysiological dysfunction and structural malformation, generate a epileptogenic developmental substrate. The coexistence of these two pathogenic branches distinguishes *SCN3A* encephalopathy from purely channelopathic epilepsies and suggests that successful gene therapy may need to address both electrophysiological and structural abnormalities. (D) Integrated clinical spectrum. Convergence of neuronal hyperexcitability and cortical malformation results in a spectrum that includes early-onset seizures, often drug-resistant epilepsy, DEE, developmental impairment, intellectual disability and structural brain abnormalities such as polymicrogyria. Selected GOF cases may benefit from sodium channel blockers, although treatment response is variant-dependent and is therefore not presented as a uniform precision therapy. Therapeutic development for *SCN3A* remains at an early exploratory stage; to date, no ASO-, siRNA- or gene-editing strategy specifically targeting *SCN3A* has entered formal preclinical validation, reflecting the genetic and phenotypic complexity described previously. Solid arrows indicate causal or pathogenic progression; dashed arrows indicate secondary or lower-emphasis links. DEE, developmental and epileptic encephalopathy; GOF, gain of function; LOF, loss of function; MCD, malformation of cortical development; NDD, neurodevelopmental disorder; *SCN3A*, sodium voltage-gated channel alpha subunit 3.

encode the Kv7.2 and Kv7.3 potassium channels, which assemble into Kv7.2/Kv7.3 heteromeric complexes and mediate the M current. These channels serve a key role in maintaining the resting membrane potential and suppressing neuronal hyperexcitability. LOF mutations in these genes are among the most common monogenic causes of neonatal epilepsy, with clinical phenotypes ranging from benign familial neonatal epilepsy to early infantile epileptic encephalopathy associated with severe developmental impairment (11).

Gene supplementation strategy. AAV-mediated delivery of wild-type *KCNQ2* or KCNQ3 cDNA may restore potassium channel function. Electrophysiological studies have demonstrated that channel dysfunction resulting from *KCNQ2/3* mutations can be partially rescued by supplementation with the corresponding wild-type gene (10,78,79). However, the coding regions of *KCNQ2* and *KCNQ3* are relatively large (~2.5 and 2.4 kb, respectively). Although each gene fits within the packaging capacity of a single AAV vector, co-delivery of both genes remains challenging (80). Consequently, despite encouraging proof-of-concept data, KCNQ2/3 gene supplementation remains at the preclinical stage.

Small-molecule activator strategy. Enhancing residual Kv7 channel activity with small-molecule compounds represents a more advanced clinical approach. The first-generation drug retigabine was previously approved for adults with refractory epilepsy but was withdrawn because of long-term adverse effects, including skin and retinal pigmentation. Next-generation Kv7 channel activators, such as XEN1101 (68) and RL648_81 (81), are under development and exhibit greater selectivity, improved brain penetration and enhanced safety profiles. XEN1101 has demonstrated favourable efficacy in a Phase IIb clinical trial (82). As numerous Kv7-channel activators have progressed into human clinical studies, this therapeutic strategy is currently in clinical development (83,84).

CRISPRa strategy. In the future, CRISPRa-mediated activation of endogenous KCNQ2/3 expression may provide a precise, durable way to restore channel function. However, this approach remains at an early proof-of-concept stage.

mTOR pathway-associated epilepsy-synergy between gene repair and pharmacological inhibition. The mTOR signalling pathway serves a central role in regulating cell growth, metabolism and synaptic plasticity. Aberrant activation of this pathway, termed mTORopathy, can lead to cortical developmental abnormalities and refractory epilepsy, with mutations in *TSC1*, *TSC2* and DEP domain-containing protein 5 (*DEPDC5*) among the best-characterised examples (85,86). Fig. 4 illustrates the pathogenic mechanisms and precision therapeutic strategies associated with mTOR-pathway-associated epilepsy.

Gene repair strategy. CRISPR/Cas9-mediated correction of pathogenic variants in *TSC1*, *TSC2* or *DEPDC5* can effectively suppress mTORC1 activity, as reflected by reductions in pS6 levels, in animal models and iPSC-derived brain organoids, thereby improving neuronal development and electrophysiological abnormalities (87). Guo *et al* (12) reported successful CRISPR-mediated correction of pathogenic *TSC2* variants in iPSCs derived from patients with TSC type 2. Despite these promising findings, gene-repair approaches for mTOR-pathway-associated epilepsy remain at the preclinical stage.

mTOR inhibitors (rapalogs). mTOR inhibitors such as everolimus pharmacologically suppress mTORC1 activity and have been approved by the Food and Drug Administration for the treatment of *TSC*-associated epilepsy. The Phase III EXIST-3 trial demonstrated that everolimus notably reduced seizure frequency and decreased brain lesion burden, while offering the advantages of oral administration, rapid onset of action and reversibility (88). However, sustained efficacy requires long-term treatment, some patients exhibit reduced responsiveness over time, and therapy may be associated with immunosuppressive and metabolic adverse effects. Nevertheless, everolimus represents the most clinically advanced therapeutic approach targeting the mTOR pathway and is currently approved for the treatment of TSC-associated epilepsy.

Combination strategies. Each therapeutic approach has inherent limitations. Consequently, synergistic strategies have been proposed, such as combining low-dose rapamycin with CRISPR-based correction of *TSC* mutations to reduce synaptic hyperactivity while enhancing the stability of gene-expression regulation or using pharmacological therapy to control seizure activity and thereby create a therapeutic window for subsequent gene editing (12,89). Such combination approaches may be particularly promising for the treatment of childhood developmental epilepsies.

Emerging synapse-associated genes: GRIN2A, GABRG2 and STXBP1. Epilepsy genetics research has expanded beyond ion channel genes to include genes involved in synaptic function and neurotransmitter regulation. *GRIN2A*, *GABRG2* and *STXBP1* exemplify the key contribution of synaptopathies to epilepsy pathogenesis.

GRIN2A. *GRIN2A* encodes the glutamate receptor ionotropic NMDA 2A subunit of the NMDA-type glutamate receptor, and pathogenic variants are closely associated with epileptic encephalopathies, including developmental language disorder and electrical status epilepticus during sleep (90). Different mutation types can either enhance or impair NMDA receptor function. GOF variants increase calcium permeability and persistent current, supporting the potential use of NMDA receptor antagonists such as memantine. By contrast, LOF variants may be amenable to CRISPRa-mediated activation of endogenous GRIN2A expression to restore physiological pathway activity (91). However, these therapeutic approaches remain at an early exploratory stage, with limited evidence currently available beyond proof-of-concept studies.

GABRG2. GABRG2 encodes the γ2 subunit of the γ-aminobutyric acid sub-type A receptor, an important component of inhibitory neurotransmission. Pathogenic variants frequently result in abnormal subunit localisation, splicing dysregulation or impaired membrane trafficking, ultimately reducing inhibitory currents while inducing endoplasmic reticulum stress and protein misfolding (92). Therapeutic strategies under investigation include chemical chaperones (93), ASO-mediated splice correction and CRISPRa-mediated enhancement of gene expression (40), all of which have demonstrated efficacy in experimental model systems. However, these approaches remain at the preclinical stage of development and have not yet advanced to clinical evaluation.

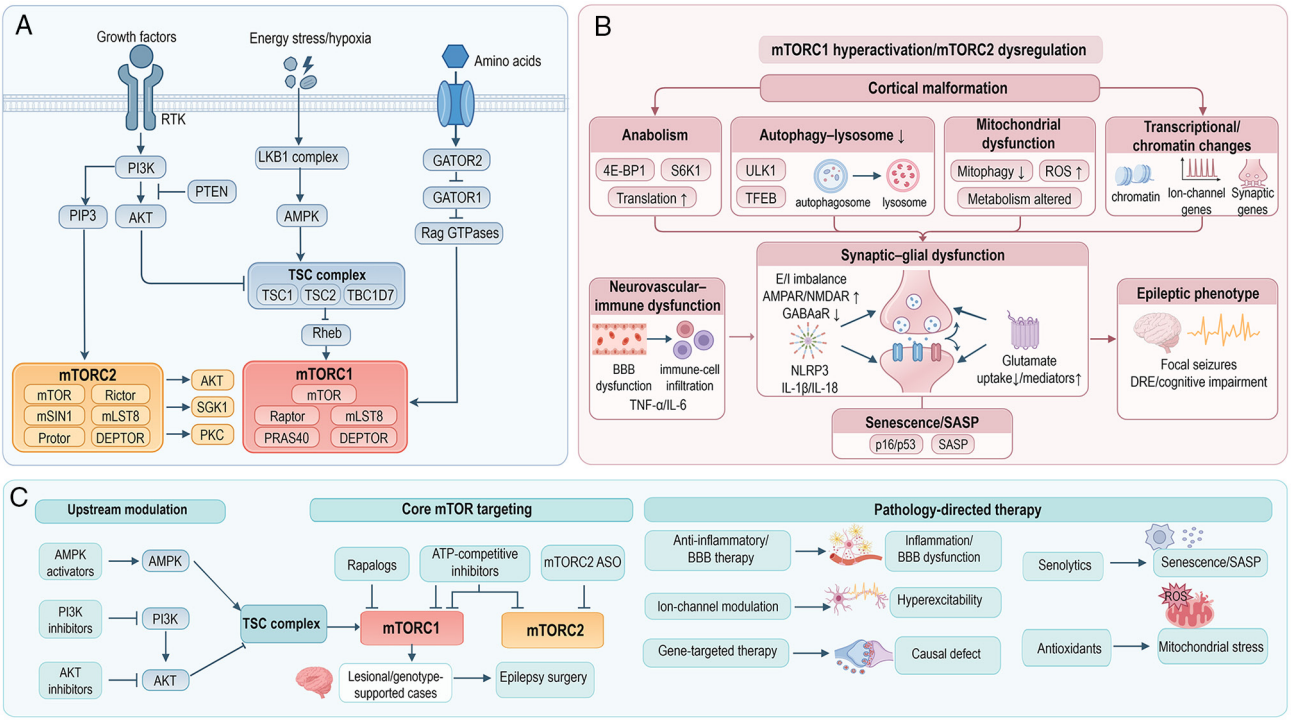


Figure 4. Pathogenic mechanisms and precision therapeutic strategies in mTOR pathway-associated epilepsy. This figure illustrates the complex regulatory architecture of the mTOR signalling pathway, the pathogenic mechanisms associating mTOR dysregulation to epileptogenesis and the spectrum of precision therapeutic strategies, ranging from clinically established to emerging and preclinical approaches, that target different nodes within this pathway. (A) Upstream inputs and core regulatory architecture. Growth factor signalling activates the PI3K-AKT axis, which PTEN restrains. Energy stress is sensed through the LKB1-STRAD-MO25-AMPK axis, whereas amino acid availability is detected through the GATOR2-GATOR1-Rag pathway. The TSC complex inhibits Rheb and thereby restrains mTOR complex 1 (mTORC1). mTOR signalling is organised into two functionally distinct complexes: mTORC1, which regulates cell growth and metabolism, and mTOR complex 2 (mTORC2), which modulates cytoskeletal organisation and cell survival. The complexity of this regulatory network, with multiple inputs converging on two distinct complexes, creates both therapeutic opportunities (multiple intervention nodes) and challenges (the potential for compensatory signalling and off-target effects). (B) Pathogenic mechanisms associating mTOR dysregulation to epileptogenesis. mTORC1 hyperactivation and mTORC2 dysregulation promote anabolic drive, impaired autophagic-lysosomal flux, mitochondrial dysfunction, metabolic remodelling and transcriptional and chromatin dysregulation. These abnormalities converge on synaptic and glial network dysfunction, including altered vesicle cycling, excitatory-inhibitory imbalance, astrocyte dysfunction and microglia-driven neuroinflammation. Downstream inflammatory cascades, including NLRP3 inflammasome activation, inflammatory mediators (IL-1β, IL-18, TNF-α and IL-6), and BBB dysfunction with immune-cell infiltration, further amplify epileptogenesis. Cellular senescence, characterised by a SASP, adds another layer of chronic neuroinflammation that sustains the epileptogenic state. Developmental cortical malformation is shown as an upstream structural substrate that predisposes to network hyperexcitability and seizure generation. (C) Precision therapeutic strategies and major intervention nodes. Therapeutic approaches are grouped into established and clinically actionable; investigational and early clinical; and emerging and preclinical categories, and mapped to upstream signalling nodes, core mTOR complexes or downstream disease mechanisms. Established and clinically actionable strategies include everolimus (an mTORC1 inhibitor) for TSC-associated seizures and epilepsy surgery in selected lesional or genotype-supported cases. Investigational and early clinical approaches encompass upstream pathway modulators (AMPK, PI3K and AKT inhibitors or modulators) and core pathway inhibitors (rapamycin analogues and ATP-competitive mTOR inhibitors). Emerging and preclinical strategies include mTORC2-targeting ASOs, senolytics, anti-inflammatory and BBB-stabilising approaches, ion-channel modulation and gene-targeted therapies (such as CRISPR-based correction of TSC1/TSC2 mutations). Importantly, therapeutic targeting of the mTOR pathway is not one-size-fits-all: Rapamycin analogues act primarily on mTORC1, whereas ATP-competitive inhibitors target both mTORC1 and mTORC2, offering broader suppression but potentially greater toxicity. The diversity of intervention nodes, from upstream signalling inputs to downstream inflammatory and metabolic effectors, enables a multi-tiered therapeutic approach, although the optimal combination and sequencing of these strategies remain to be determined. Solid arrows indicate activation or causal flow; T-shaped lines indicate inhibition; dashed arrows indicate indirect, emerging or lower-evidence links. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AMPK, AMP-activated protein kinase; AKT, AKT serine/threonine kinase; ASO, antisense oligonucleotide; BBB, blood-brain barrier; CRISPR, clustered regularly interspaced short palindromic repeats; GABA, γ-aminobutyric acid; GATOR, GAP activity towards rags complex; IL, interleukin; LKB1, liver kinase B1; mTOR, mechanistic target of rapamycin; mTORC1, mechanistic target of rapamycin complex 1; MO25, mouse protein 25; mTORC2, mechanistic target of rapamycin complex 2; NMDA, N-methyl-D-aspartate; NLRP3, NOD-like receptor family pyrin domain containing 3; PI3K, phosphatidylinositol 3-kinase; Rag, Ras-related GTP-binding protein; Rheb, Ras homolog enriched in brain; ROS, reactive oxygen species; SASP, senescence-associated secretory phenotype; STRAD, STE20-related adaptor protein; TNF-α, tumour necrosis factor; TSC, tuberous sclerosis complex.

STXBP1. *STXBP1* encodes a presynaptic vesicle-fusion protein that is important for neurotransmitter release. LOF variants reduce synaptic vesicle exocytosis, impair synaptic transmission and neural network development and are associated with early-onset epileptic encephalopathies, including Ohtahara syndrome and West syndrome (94,95). *STXBP1* mouse models recapitulate a number of key disease features, including recurrent seizures and cognitive impairment. Preclinical studies have shown that both ASO-mediated upregulation of *STXBP1* expression and AAV-mediated gene replacement can markedly reduce seizure burden and improve behavioural outcomes (94,95), supporting the potential of gene-targeted therapies for STXBP1-associated disorders. Consistent with these advances, Konishi *et al* (96) extended the base-editing paradigm to protein conformational epilepsies by demonstrating personalised ABE-mediated correction

Table IV. Summary of strategies for synapse-associated genes.

Gene	Protein function	Major mutational effect	Therapeutic strategy	Translational status
Glutamate ionotropic receptor NMDA type subunit 2A	NMDA receptor subunit	GOF and LOF	Antagonists (GOF)/ CRISPRa (LOF)	Early exploration
γ-aminobutyric acid type A receptor subunit γ2	γ-aminobutyric acid receptor subunit	LOF (reduced inhibition)	Chemical chaperones, ASOs and CRISPRa	Preclinical
Syntaxin binding protein 1	Synaptic vesicle fusion	LOF	ASO upregulation and adeno-associated virus gene supplementation	Preclinical

GOF, gain-of-function; LOF, loss-of-function; CRISPRa, clustered regularly interspaced short palindromic repeats activation; ASO, antisense oligonucleotides; NMDA, N-methyl-D-aspartate

of a pathogenic serpin family I member 1 variant causing familial encephalopathy with neuroserpin inclusion bodies, a progressive myoclonic epilepsy (96). Despite these promising results, *STXBP1*-targeted gene therapies remain in preclinical development. The therapeutic strategies and translational status of representative synapse-associated epilepsy genes are summarised in Table IV.

6. Challenges and limitations

Although gene therapy for epilepsy has made substantial progress across multiple areas of research, its clinical translation continues to face complex technical and regulatory challenges. This section summarises five major bottlenecks.

Genetic heterogeneity and mechanistic complexity. The marked genetic heterogeneity of epilepsy necessitates individualised, mechanism-based therapeutic strategies (97). GOF mutations are generally more amenable to gene-silencing approaches, whereas LOF mutations are more likely to require gene supplementation, gene activation or genome-editing repair. Although this principle of mechanism-matched precision therapy is conceptually attractive and has shown considerable promise in preclinical models, several challenges limit its clinical implementation.

Incomplete variant annotation. The pathogenic significance of numerous rare variants remains uncertain, and some variants exhibit mixed functional effects rather than fitting neatly into LOF or GOF categories. Recent systematic assessments of *in silico* prediction tools have highlighted both the potential and the limitations of current computational methods for classifying epilepsy-associated variants (98).

Phenotypic overlap. Different mutations within the same gene may result in markedly distinct clinical phenotypes. For example, *SCN2A*-associated disorders may arise from either LOF or GOF variants, increasing uncertainty in therapeutic strategy selection.

Non-ion-channel mechanisms. Certain forms of refractory epilepsy involve mechanisms beyond ion-channel dysfunction, including neuroinflammation (99), inflammatory myeloid-cell involvement (100) and mitochondrial impairment (101). Gene therapy alone may not adequately address these pathogenic processes.

Potential solutions. Standardised workflows for functional variant validation should be established, including high-throughput electrophysiological screening and evaluation in iPSC-derived neuronal models. In addition, AI-based predictive platforms may facilitate rapid *in silico* functional classification of novel variants, thereby accelerating therapeutic decision-making.

Delivery bottlenecks: BBB and vector cargo limitations

BBB penetration. Efficient delivery of systemically administered gene therapeutics to the central nervous system remains challenging because of the restrictive nature of the BBB. Although intrathecal, intracerebroventricular and intraparenchymal administration can circumvent this barrier, these approaches are limited by invasiveness, distribution uniformity and scalability (102).

Vector cargo limitations. AAV vectors remain the most widely used delivery vehicles for central nervous system gene therapy; however, their packaging capacity of ~4.7 kb restricts the delivery of large genes, such as *SCN1A* (~6 kb) and *MECP2* (~10 kb). Dual- and triple-AAV split-vector strategies can partially overcome this limitation, but recombination efficiency and long-term safety remain areas requiring further optimisation (33,103).

Potential solutions. Next-generation AAV capsids, including engineered variants such as AAV.CAP-B10, may improve BBB penetration and cell-type-specific targeting. Alternative non-viral delivery systems, including LNPs (47) and extracellular vesicles such as exosomes (104), are also being actively investigated as potential platforms for central nervous system gene delivery. For disorders caused by mutations in large genes, strategies such as regulatory AAV approaches or CRISPRa-based transcriptional activation may be preferable because they do not require delivery of full-length cDNA.

LNPs have emerged as a promising non-viral platform for nucleic acid delivery because of their low immunogenicity, scalable manufacturing and ability to accommodate large or multiple therapeutic payloads. However, efficient BBB penetration remains an obstacle for LNP-mediated delivery

to the central nervous system. Several approaches have been developed to enhance LNP-BBB crossing.

Ligand-mediated transcytosis. Conjugation of targeting ligands that bind receptors expressed on brain endothelial cells can exploit receptor-mediated transcytosis to enhance BBB penetration. Angiopep-2, a peptide ligand for low-density lipoprotein receptor-related protein 1, has been successfully incorporated into LNP formulations to improve brain delivery. Tong *et al* (47) demonstrated that Ang-C2 LNPs enabled efficient siRNA transport across the BBB and sustained target-gene suppression for ~15 days. Similarly, ligands targeting the transferrin receptor, as well as apolipoprotein E-derived peptides, have been integrated into LNP formulations to facilitate transcytotic transport into the central nervous system.

Optimisation of surface charge and lipid composition. The physicochemical properties of LNPs, particularly surface charge and lipid composition, substantially influence their interactions with the BBB. Cationic LNPs interact more strongly with the negatively charged endothelial glycocalyx but may also increase the risk of immune activation (105). Incorporating helper lipids, such as dioleoylphosphatidylethanolamine and cholesterol derivatives, can improve membrane fusion and endosomal escape (106). Furthermore, ionisable lipids that remain neutral under physiological conditions but become positively charged within the acidic endosomal environment have been shown to enhance both BBB penetration and intracellular delivery (107).

Biomimetic strategies. Coating LNPs with cell-derived membranes, including those obtained from erythrocytes, platelets or exosomes, can prolong systemic circulation and reduce clearance by the reticuloendothelial system (108,109). Exosome-based delivery platforms are particularly attractive because they naturally traffic between the peripheral circulation and the central nervous system and may cross the BBB with minimal immunogenicity (110). However, challenges associated with large-scale production and quality control remain unresolved (111).

Focused ultrasound-mediated BBB disruption. Temporary and reversible BBB disruption achieved through focused ultrasound combined with microbubbles can enhance LNP accumulation within targeted brain regions. This approach enables spatially controlled delivery and has been reported to increase local drug and nucleic acid concentrations several-fold. Recent studies have shown that combining focused ultrasound with long-circulating nanoparticle formulations further enhances brain delivery of nucleic acids, resulting in robust, region-specific modulation of gene expression (112,113).

Microfluidic-based optimisation. High-throughput microfluidic platforms permit rapid evaluation of LNP formulation parameters, including lipid composition, mixing conditions and targeting-ligand density, facilitating the identification of formulations with improved BBB penetration (114). Increasingly, researchers are integrating machine-learning approaches into this process to predict composition-performance relationships and accelerate the development of optimised LNP platforms (115,116).

Despite these advances, LNP-mediated delivery to the brain still faces notable challenges, including limited cell-type specificity, relatively short circulation half-life and the potential need for repeated administration. Continued progress in LNP engineering, together with a more comprehensive understanding of BBB biology, is expected to substantially improve the clinical feasibility of LNP-based gene therapies for central nervous system disorders.

Off-target effects and immunogenicity. Genome-editing technologies, including CRISPR/Cas9, base editors and prime editors, may induce unintended modifications at genomic or transcriptomic sites beyond the intended target. Such off-target events can disrupt key regulatory elements or inactivate tumour suppressor genes, raising important safety concerns. Notably, even base editors, which avoid generating DNA DSBs, have been reported to produce genome-wide off-target editing events (117,118).

Multiple components of current gene-therapy platforms, including AAV vectors, Cas proteins and editing enzymes, can provoke both innate and adaptive immune responses (119). Pre-existing neutralising antibodies against AAV capsids may reduce transduction efficiency and limit the feasibility of repeat dosing. Furthermore, persistent transgene expression may induce cytotoxic T-cell responses that eliminate transduced cells, compromising therapeutic efficacy and long-term safety (120,121).

Several approaches are being explored to mitigate these risks. Developing specific editing platforms, such as high-fidelity Cas9 variants and engineered ADAR enzymes with improved target selectivity, may reduce off-target activity (117,118). In parallel, researchers are investigating biodegradable non-viral delivery systems as alternatives to viral vectors to minimise immunogenicity and facilitate repeat administration (109). Establishing standardised protocols for immune monitoring, together with evidence-based immunosuppressive strategies when appropriate, may further improve the safety and clinical applicability of gene-based therapies for epilepsy (119-121).

Paediatric safety and developmental timing. Most severe genetic epilepsies present during infancy or early childhood, necessitating therapeutic intervention during key periods of neurodevelopment. However, the paediatric nervous system is particularly sensitive to exogenous vectors and genome-editing technologies, creating distinct challenges for clinical translation (122).

Irreversibility risk. The long-term consequences of permanent genomic modification, particularly through DNA-editing approaches, remain unknown in the developing brain. Given the dynamic nature of neurodevelopment, even precise genetic interventions may have unforeseen effects that only become apparent later in life.

Developmental compensation. The timing of intervention is important. Treatment administered too early may interfere with normal developmental processes and compensatory mechanisms (123), whereas treatment delivered too late may fail to prevent the establishment of irreversible pathological neural circuits and consequently miss the optimal therapeutic window (124).

Dose design. Pharmacokinetic and pharmacodynamic characteristics differ substantially between paediatric and adult populations. As a result, effective and safe dosing strategies require age-specific modelling rather than simple extrapolation from adult data (125,126).

Vector dilution during brain growth. A further challenge unique to paediatric AAV-mediated gene therapy is the dilution of episomal AAV genomes during postnatal brain development (127). AAV vectors generally persist as extrachromosomal episomes that do not replicate during cell division. As the developing brain undergoes substantial increases in cell size, synaptic complexity and overall tissue volume, the effective vector genome copy number per cell may progressively decline (128). In humans, brain volume increases ~3-fold between birth and adulthood. This expansion may reduce transgene dosage and expression over time, potentially diminishing therapeutic efficacy (129).

Evidence from juvenile animal studies suggests that AAV-mediated transgene expression can decline relative to brain growth, reducing therapeutic benefit as development progresses (130). This issue is of particular concern for genetic epilepsies that require sustained lifelong expression of a therapeutic protein, as a dose that is effective during early childhood may become insufficient during adolescence or adulthood.

Several strategies have been proposed to address vector dilution. These include administering higher initial vector doses, although this may increase the risks of toxicity and immunogenicity; developing AAV capsids with enhanced transduction efficiency to generate higher initial genome copy numbers per cell; incorporating molecular elements that promote episomal concatemerisation or stable genomic integration, despite the safety concerns associated with integration; designing therapeutic approaches with a low efficacy threshold, such that clinical benefit can be maintained despite partial loss of expression; and implementing repeat dosing at later developmental stages, although this approach may be complicated by the development of neutralising antibodies. Collectively, these considerations highlight the importance of including long-term monitoring of transgene expression and clinical efficacy in early-phase paediatric trials to guide optimisation of dosing strategies.

Potential solutions. Reversible and controllable therapeutic modalities, such as ASOs and RNA-editing technologies, may be particularly appropriate for paediatric populations because they allow treatment adjustment as development progresses. In addition, long-term follow-up registries should be established to systematically evaluate the durability, efficacy and safety of gene therapies throughout childhood and adulthood. Finally, preclinical development programmes should incorporate juvenile animal models to assess age-dependent effects and define optimal therapeutic windows across different stages of neurodevelopment.

Regulation and accessibility of personalised therapies. As the 'one patient, one drug' paradigm continues to evolve, exemplified by the development of individualised ASOs for ultra-rare pathogenic variants, the ethical, regulatory and accessibility challenges associated with personalised therapeutics are becoming increasingly apparent (131).

High development costs. Designing, developing and manufacturing customised ASOs or genome-editing platforms for a single patient, or a small patient population, involves substantial financial and logistical burdens. These therapies often lack a clear commercial pathway, limiting investment and large-scale implementation.

Uncertain regulatory pathways. Conventional regulatory frameworks largely rely on evidence from randomised controlled trials. Such approaches are often impractical or impossible for personalised N-of-1 therapies, creating an urgent need for alternative regulatory models that can evaluate safety and efficacy in ultra-small patient populations.

Equity and accessibility concerns. Personalised medicines may inadvertently exacerbate disparities in healthcare access and resource allocation. The substantial costs and specialised infrastructure required for development may limit access to a small number of patients and healthcare systems, raising important questions about fairness and healthcare equity.

Potential solutions. Accelerated regulatory pathways and shared data repositories for personalised nucleic acid therapeutics may enable more efficient development and evaluation of these therapies (132). Greater involvement of academic institutions, public-sector organisations and non-profit foundations may help support therapeutic development for rare disorders that lack commercial incentives. In parallel, implementing standardised pipelines for the design, manufacturing, quality control and clinical evaluation of individualised ASOs could improve efficiency, reduce costs and promote broader accessibility. Collectively, these measures may help create a sustainable framework for translating personalised genetic therapies from experimental concepts into clinically accessible treatments.

7. Future directions

Gene therapy for epilepsy is at an important stage in the transition from experimental validation to clinical application. Future development will focus on four major directions.

Personalized therapy. With advances in high-throughput sequencing and the expansion of mutation databases, the 'one patient, one drug' approach is emerging for rare mutations, including those affecting *SCN1A* and *SCN2A* (133). ASOs, siRNAs and gene-editing tools designed for individual pathogenic variants have demonstrated promising efficacy in animal models of neurological disorders and in *ex vivo* studies using patient-derived cells (44,132). As synthesis platforms and delivery technologies continue to mature, a closed-loop process encompassing rapid diagnosis, sequence design, drug synthesis and clinical validation is becoming increasingly feasible. Collaborative initiatives such as the N=1 Collaborative are further promoting data sharing and standardisation for individualised nucleic acid therapies (132).

Combination intervention strategies. Combining gene therapy with small-molecule drugs, ASOs or protein stabilisers may provide complementary benefits through structural repair, functional compensation and pathway stabilisation. Representative examples include TSC-associated epilepsy. By combining CRISPR-based repair with mTOR inhibitors, such as low-dose rapamycin, may improve therapeutic durability while reducing adverse effects associated with monotherapy (12). For GOF epilepsy, combining ASO-mediated gene silencing with sodium-channel blockers may produce synergistic anti-seizure effects. For LOF epilepsy, combining CRISPRa-mediated activation with protein stabilisers may increase overall

functional protein production. Previous studies have shown that CRISPRa (134) and RNA splicing modulation (135) each offer distinct advantages in enhancing gene expression and limiting toxicity. Their combination may therefore provide synergistic benefits, although comprehensive validation in both *in vitro* and *in vivo* systems remains necessary.

AI-assisted design. One of the most immediate applications of AI in epilepsy gene therapy is addressing the challenge of variant functional classification (as aforementioned). When clinical sequencing identifies a novel variant in *SCN1A*, *SCN2A* or another epilepsy-associated gene, AI-based tools can provide rapid functional predictions to support therapeutic strategy selection. Machine learning models trained on large-scale mutagenesis and functional datasets, including deep mutational scanning and electrophysiological recordings, can predict whether a missense variant is likely to result in LOF or GOF based on sequence, structural and evolutionary features. For example, AlphaMissense, which integrates protein structure prediction with evolutionary conservation, has demonstrated state-of-the-art performance in distinguishing pathogenic from benign variants. Likewise, specialised tools such as Variant Site, Screening for Non-Acceptable Polymorphisms 2 and Meta Recurrent Neural Network can generate functional consequence predictions within minutes of variant identification.

When integrated with clinical phenotype information and *in vitro* validation pipelines, these AI-based predictions can substantially accelerate therapeutic decision-making. For example, a predicted GOF mutation in *SCN8A* would support consideration of ASO-mediated silencing, whereas a predicted LOF mutation in *SCN1A* would favour ASO-mediated upregulation or regulatory AAV approaches. This rapid triage capability has the potential to reduce the interval between genetic diagnosis and treatment selection from weeks or months to only a few days, a particularly important advantage for infants with severe and rapidly progressive developmental and epileptic encephalopathies. Beyond variant classification, AI tools are also being developed to optimise gRNA design for CRISPR-based applications, predict pegRNA sequences for prime editing (136), identify novel AAV capsids through machine-learning approaches (22,137) and select ASO target sites with high on-target activity and minimal off-target effects. These capabilities are expected to serve an important role in the future development of personalised therapies.

Integrated AI decision platforms may ultimately enable an automated workflow spanning mutation diagnosis, therapeutic design and treatment recommendation, thereby accelerating the implementation of precision medicine.

Development of translational research platforms. Standardised and high-fidelity translational research platforms provide a key bridge between basic research and clinical application. iPSC-derived neurons enable the reproduction of electrophysiological phenotypes within patient-specific genetic backgrounds and have become valuable tools for both drug screening and gene therapy validation. For example, an iPSC-derived neuronal model of *SCN3A* encephalopathy successfully recapitulated abnormal sodium currents and was subsequently used for therapeutic screening (75). Beyond 2D cellular systems, 3D brain organoids more closely mimic the structural and functional complexity of the developing human brain. These models can reproduce network dysfunction and cortical developmental abnormalities associated with pathogenic mutations, providing a physiologically relevant platform for evaluating the cell-type specificity and long-term safety of gene therapies.

Animal models continue to serve a central role in translational development. Humanised mouse and rat models carrying pathogenic human variants facilitate the assessment of vector delivery efficiency, behavioural outcomes and immunological safety under *in vivo* conditions. At the highest level of preclinical validation, non-human primate models remain indispensable for clinical translation because they permit evaluation of AAV capsid distribution throughout the brain, long-term safety of gene-editing technologies and treatment-associated immunogenicity. Together, these complementary platforms provide an integrated translational pipeline that supports the progression of gene therapies from mechanistic investigation to clinical implementation.

8. Conclusions

Gene therapy for genetic epilepsy is at an important juncture in its transition from theoretical validation to clinical application. Using the functional consequences of mutations (LOF/GOF) as the primary framework for therapeutic decision-making, the present review systematically outlines the applicable scenarios, translational maturity and major bottlenecks associated with five technological approaches. The main conclusions are as follows.

LOF and GOF mutations require fundamentally different intervention strategies. LOF epilepsies are suited to expression-enhancing approaches, such as gene supplementation, regulatory AAVs and CRISPRa, whereas GOF epilepsies require gene-silencing technologies, including ASOs, siRNA and CRISPRi. This principle of matching strategy to mechanism underpins rational translational decision-making and is a central contribution of the present review.

The five therapeutic platforms exhibit markedly different levels of translational and clinical maturity. ASOs and regulatory AAVs, supported by established delivery systems and controllable safety profiles, have progressed furthest towards clinical translation (such as the Phase III trial of STK-001 and the Phase I trial of ETX101). Endogenous activation technologies such as CRISPRa provide a genome-editing-independent alternative for LOF epilepsies but remain at the stage of preclinical optimisation. Although DNA and RNA editing technologies offer the potential for durable disease modification and, in some cases, one-time therapeutic intervention, further evidence regarding delivery efficiency, specificity and long-term safety is required.

Clinical translation faces five major systemic bottlenecks: Genetic heterogeneity and mechanistic complexity; BBB penetration and vector cargo limitations; off-target effects and immunogenicity; paediatric safety and developmental timing; and the regulation and accessibility of personalised therapies. Addressing these challenges will require coordinated advances in molecular genetics, neuroscience, vector engineering, clinical medicine, regulatory science and bioinformatics.

Future progress in the field is likely to be driven by four major areas: Personalised therapy, combination intervention strategies, AI-assisted design and standardised translational research platforms. Through the deep integration of these

approaches, epilepsy treatment may shift from symptom control to mechanistic correction.

A major contribution of the present review is a mutation function-guided framework that aligns pathogenic mechanisms with appropriate therapeutic modalities, facilitating rational therapeutic selection in genetic epilepsy. By systematically mapping LOF and GOF mutations to corresponding intervention strategies, expression enhancement for LOF and gene silencing for GOF, and by providing a comparative evaluation of five core technological platforms, this framework offers a practical decision-support tool for clinicians and researchers navigating the increasingly complex landscape of gene-based epilepsy therapies.

Despite these advances, several important knowledge gaps remain. The relationship between genotype and phenotype in genetic epilepsy is often non-linear, and numerous variants exhibit context-dependent functional effects that complicate therapeutic decision-making. The long-term safety of persistent gene expression or editing in the developing brain has not been systematically evaluated. The optimal timing of intervention, balancing early treatment to prevent developmental deterioration against the risks associated with intervention in immature neural circuits, remains unclear. Furthermore, longitudinal investigation is needed to assess the durability of therapeutic effects and the potential loss of efficacy from vector dilution, immune responses or epigenetic silencing. Addressing these gaps will require coordinated efforts in preclinical modelling, long-term follow-up registries and iterative refinement of therapeutic platforms.

In summary, the LOF/GOF decision framework and technology-specific evaluation system proposed in the present review are intended to provide a reference for selecting gene therapy strategies for genetic epilepsy while highlighting the key gaps that must be addressed to advance the field. With continued optimisation of delivery technologies, editing tools and regulatory systems, gene therapy has the potential to become an increasingly important component of future precision treatment strategies for selected forms of genetic epilepsy, ultimately benefiting a growing proportion of patients affected by these devastating disorders.

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Authors' contributions

RQ was responsible for conceptualization of the present review, writing the original draft, visualization, formal analysis, data curation, literature collection and validation. XH, WJ, JW and MZ contributed to literature collection, data curation and validation, and reviewing and revision of the manuscript for important intellectual content. ZZ was responsible for reviewing and editing the manuscript and funding acquisition. DM was responsible for conceptualization, supervision, review and editing the manuscript and funding acquisition. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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