Trends in Molecular Medicine

CellPress

Review

Precision medicine for sodium channelopathy-related autism and epilepsy

Morgan Robinson[1,2,3], Kyle Wettschurack[1,3], Manasi S. Halurkar[1,3], Xiaoling Chen[1,3], Zaiyang Zhang[1,3], Jingliang Zhang[1,3], and Yang Yang[1,3,*]

Precision medicines for monogenic brain disorders are rapidly advancing. Voltage-gated sodium channel (VGSC) genes are the leading monogenic cause of severe epilepsy and profound autism spectrum disorder (ASD), most notably *SCN1A*, *SCN2A*, *SCN3A*, and *SCN8A*. Recent advances in animal and human induced pluripotent stem cell (hiPSC) disease models provide a powerful platform for advancing precision medicines. Thanks to the genomic revolution, many gene therapies are in preclinical studies and clinical trials for VGSC-related diseases, including viral vector gene replacement, clustered regularly interspaced short palindromic repeats (CRISPR) base editing, prime editing, and genetic modulation strategies including antisense oligonucleotides, engineered tRNAs, and CRISPR activation/interference (CRISPRa/i). This review highlights the latest advances in disease modeling and next-generation therapeutic development to advance precision medicine for VGSC-related brain disorders.

Highlights

Animal models of voltage-gated sodium channel (VGSC)-related epilepsy and autism reveal insights into disease mechanisms and provide phenotypes to test precision medicines.

Human induced pluripotent stem cell - derived models are rapidly advancing to include 2D cultures, 3D organoids and assembloids, and chimeric mouse models to test therapies in human cells with human gene sequences.

Gene replacement therapies using viral vectors are promising for the treatment of VGSC haploinsufficiency in preclinical trials.

Advances in CRISPR-based platforms (base and prime editing) represent promising gene correction therapies for monogenic brain disorders.

Modalities that modulate gene and protein expression, such as antisense oligonucleotides, engineered tRNAs, CRISPRa/i, and CRISPR epigenetic regulation are evolving rapidly.

Challenges with targeted brain delivery remain one of the largest hurdles in moving forward.

Precision medicine for treating monogenic brain disorders

The precision medicine era is rapidly emerging and the promise of the genomic revolution is finally being realized through treatments that are tailored to patient-specific genetic sequences. While precision therapies have been transformative in other fields [1], central nervous system (CNS) disorders are lagging behind, particularly because of the enormous complexity of the brain, diverse cell types, and the restrictive blood–brain barrier (BBB) [2]. However, the burden of rare *de novo* monogenic CNS disorders is high, and has an estimated global incidence of 1 in 304 individuals [3]. Most rare monogenic CNS disorders do not have disease-modifying pharmaceutical interventions, but may be particularly amenable to gene therapies because of their monogenic nature [2,4]. Thus, there are tremendous opportunities to advance precision therapies for these disorders in the coming decades.

A large subset of neurodevelopment disorders, in particular developmental and epileptic encephalopathy (DEE) and autism spectrum disorder (ASD), are caused by mutations in voltage-gated sodium channel (VGSC) genes, including *SCN1A*, *SCN2A*, *SCN3A*, *SCN8A*, and *SCN1B* (*SCN2A* 2D and 3D structures are shown in Figure 1A,B). These VGSC-related disorders also involve motor, sensory, and visual impairments [5,6]. VGSCs are responsible for the initiation and propagation of action potential (AP) firing, and also regulate dendritic membrane potentials and AP backpropagation [7,8]. Each VGSC differs in its cell type expression patterns, distribution, and subcellular localization (Figure 1C). They also exhibit unique biophysical properties and contribute to AP firing properties differently (Figure 1D). Mutations can cause loss-of-function (LoF), gain-of-function (GoF), or mixed-function effects on VGSCs, and alter neuronal and circuit behavior in ways that need to be considered for therapeutic development [4,6,9–11]. In any case, efficient and safe precision genome editing to directly correct disease-causing mutations would be an optimal solution. Several recent reviews on the physiology, mechanisms, and clinical advances

[1]Borch Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, Purdue University, West Lafayette, IN, USA
[2]Department of Chemical Engineering, College of Engineering, Purdue University, West Lafayette, IN, USA
[3]Purdue Institute for Integrative Neuroscience, Purdue University, West Lafayette, IN, USA

*Correspondence:
yangyang@purdue.edu (Y. Yang).

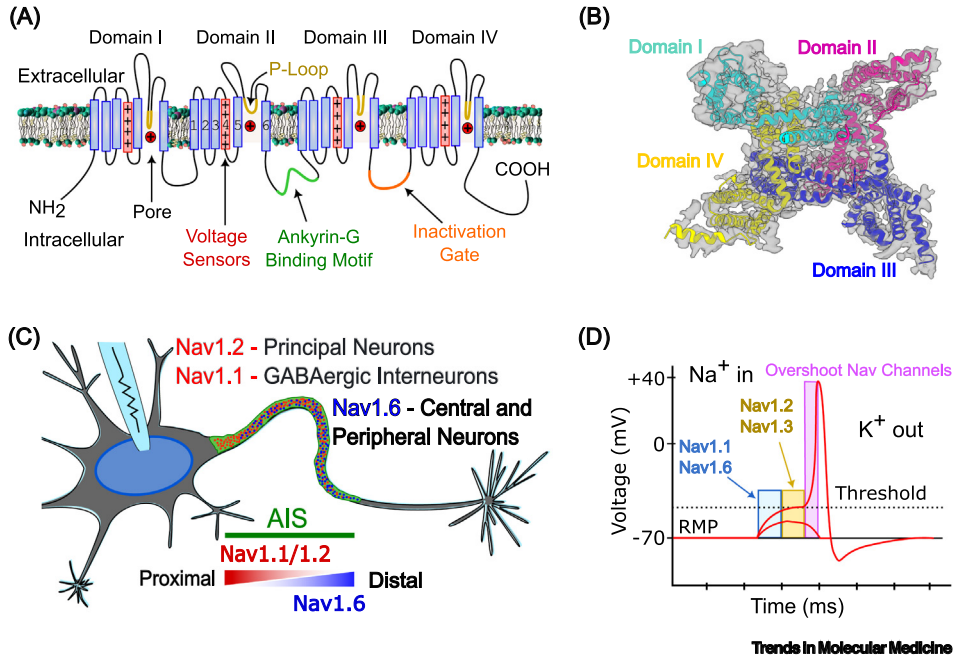

Figure 1. Voltage-gated sodium channel (VGSC) structure and function. (A) 2D representation of the SCN2A VGSC α subunit. VGSCs are a family of structurally conserved membrane proteins that contain four major domains, each comprising six membrane-spanning α-helices. VGSCs have a conserved ankyrin-G binding motif between domains II and III, which is essential for trafficking to the axon initial segment and nodes of Ranvier. The inactivation gate is found between domains III and IV. Not shown are the VGSC β subunits which modulate channel properties and play other important roles in neuronal physiology. (B) A 3D cryo-electron microscopy (cryo-EM) structure of Nav1.2 complexed with μ-conotoxin KIIIA generated from entry EMD-9780 in the Electron Microscopy Data Bank (EMDB) [135]. (C) Subcellular localization of the predominant VGSCs (SCN1A, SCN2A, and SCN6A) found in adult principal excitatory and inhibitory neurons in the nervous system. VGSCs are mainly localized within the axon initial segment (AIS) and nodes of Ranvier, where action potential firing is initiated and propagated, although they are also expressed in the somatodendritic compartment. (D) Diagram showing the action potentials of low-threshold activation channels (Nav1.1, Nav1.6) and higher-threshold channels (Nav1.2, Nav1.3) that reflect their distinct roles in neuronal excitability. Abbreviations: AIS, axon initial segment; P-loop, pore loop; RMP, resting membrane potential.

in managing VGSC disorders related to epilepsy and autism can be found elsewhere [4,6,9,10,12], while this review will focus on trends in precision genomic medicine for these neurodevelopmental disorders.

To advance precision medicine through the preclinical phase, there are two essential pillars we will discuss in this article: (i) the establishment of disease models, including animal and human cell-based models suitable for testing genetic medicines, and (ii) the development of target-specific and diverse interventions that can be experimentally tested in these disease models.

Disease models of VGSC-related monogenic epilepsy and ASD

The preclinical therapeutic development pipeline relies on *in vitro* and *in vivo* disease models that recapitulate human disease phenotypes. Disease phenotypes and mechanisms related to brain disorders can be studied using a variety of methods, including functional electrophysiology, advanced imaging, next-generation sequencing, and behavioral assays (Figure 2). In addition to animal and cell studies, *in silico* models are also useful for studying VGSC contributions to neuron and network mechanisms [13–15]. Recent advances in animal and human disease models provide a means to advance therapy development toward precision medicine.

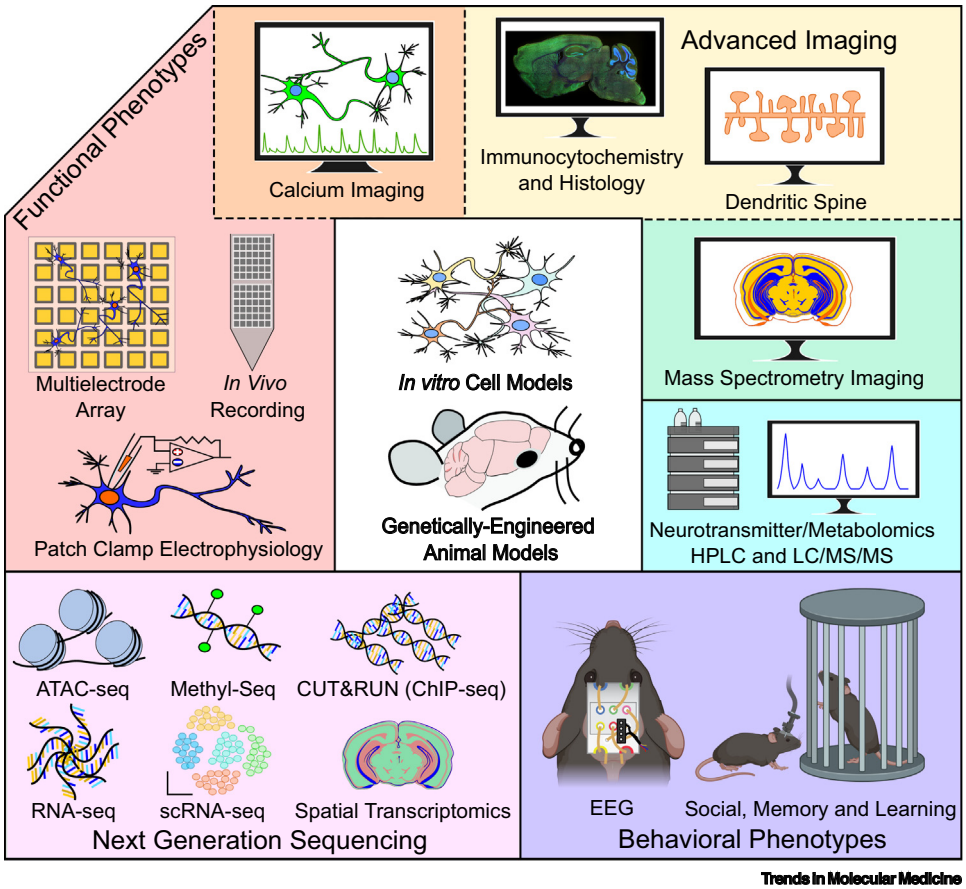

Figure 2. Phenotyping methods to test precision medicine for brain disorders. (Left) Functional neuronal phenotyping for epilepsy and autism can include a variety of *in vitro*, *ex vivo*, *or in vivo* electrophysiology recordings. Calcium imaging bridges the gap between functional assays and advanced imaging. (Top) Immunocytochemistry and immunohistology to quantify anatomical changes in brain structure, and high-resolution morphological analyses of neurons and neuronal components are important readouts for characterizing central nervous system (CNS) disorders (middle panel). (Right) Mass spectrometry (MS) imaging and neurotransmitter/metabolomics can provide important neurochemistry readouts for quantifying systems-level dysfunction. (Lower right) Behavioral phenotypes can reveal clinically relevant correlations that underlie neurological diseases and are useful for evaluating precision medicines. (Lower left) Finally, next-generation sequencing can provide important readouts for identifying system-level dysfunction and identifying novel compensatory pathways that could present new opportunities for precision medicine interventions. Abbreviations: ATAC-seq, assay for transposase-accessible chromatin sequencing; ChIP-seq, chromatin immunoprecipitation and deep sequencing; CUT&RUN, cleavage under targets and release using nuclease; EEG, electroencephalography; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; scRNA-seq, single-cell RNA sequencing.

Animal models reveal phenotypes that can be used to evaluate precision medicines

Rodent models have been the cornerstone of disease phenotyping, understanding disease mechanisms, and testing new therapeutic modalities (Table 1 lists seminal and recent VGSC-related animal models). These models allow the identification of the mechanistic basis of channelopathy-related autism and epilepsy (Box 1). Interestingly, animal strain-specific phenotypes can be drastically different, and some genetic backgrounds exhibit no discernible phenotypes while others have severe phenotypes [16,17]. Advanced genetic tools have permitted the generation of conditional knock-out and knock-in models using recombinase (Cre/*loxP*) systems that enable cell type-specific gene manipulation. These models refine our

Table 1. Select animal models of VGSC pathologies related to epilepsy and ASD

Gene	Species	Genotype	Year	Channel function	Phenotype	Refs
SCN1A	Mouse	*Scn1a* knockout (Δexon26)[a]	2006	LoF	DS	[19]
		Floxed stop *Scn1a**A1783V	2019	LoF	DS	[22]
		Scn1a-R1648H	2010	LoF	DS	[20]
		*Scn1a**K1270T, K1259T	2021	LoF	GEFS+	[21]
	Rat	*Scn1a* knockout	2023	LoF	DS	[24]
	Rabbit	*Scn1a* knockout	2022	LoF	DS	[23]
SCN2A	Mouse	*Scn2a* knockout (NaChαII) (Δexon2)[b]	2000	LoF	+/– Mild behavioral impairment; –/– lethality	[28]
		Scn2a-p.GAL879-881QQQ (Q54)[c]	2001	GoF	Seizures	[35]
		Scn2a-p.R102Q	2024	LoF	decreased vocalization	[33]
		Scn2a gene trap	2020	LoF	Strong ASD-related social deficits	[29]
		Scn2a-p.Δ1898/+	2021	LoF	increased social behavior, hyperactivity	[32]
		Scn2a-p.R1882Q	2021	GoF	Seizure, SUDEP	[36]
		Scn2a-p.K1422E	2022	Mixed Function	Altered seizure susceptibility	[37]
	Rat	LE-*Scn2a*^{em1Mcwi}	2021	LoF	increased social behavior	[34]
SCN3A	Mouse	*Scn3a*^{Gt}	2017	LoF	Seizure susceptible	[39]
SCN8A	Mouse	*Scn8a*^{MED} (frameshift knockout)[d]	1968	LoF	Motor end-plate disease	[44]
		Scn8a-p.N1768D	2017	GoF	Seizures	[47]
		Scn8a-p.R1872W	2019	GoF	Seizures, SUDEP	[50]
		Scn8a-p.R1620L	2021	GoF	Seizures	[49]
SCN1B	Mouse	*Scn1b*-null	2004	LoF	Seizures	[53,55]

[a]*Scn1a* knockout (Δexon26) was the first knockout LoF model of DS.
[b]*Scn2a* knockout (NaChαII) (Δexon2) was the first knockout *Scn2a* model.
[c]*Scn2a*-p.GAL879-881QQQ (Q54) is a non-human variant GoF model.
[d]*Scn8a*^{MED} mice were identified in 1968; later, in 1995, a frameshift knockout of the *Scn8A* gene was shown to be the cause of the MED phenotype.

understanding of disease mechanisms by pinpointing the specific contributions of different cell types. By contrast, global knock-in of patient-specific mutations mimics the genetic and functional characteristics of the human condition and enables direct translational research, while

Box 1. The complex roles of VGSCs in the mechanisms of epilepsy and ASD.

The concepts of GoF and LoF have greatly advanced our understanding of VGSC mutations and helped guide clinical interventions [6,25]. Although the LoF/GoF spectrum is useful in relating sodium channel properties to patient phenotypes, this paradigm may oversimplify the predictions of a mutation's functional effects on neuronal and network activities, due to compensatory mechanisms at the gene regulation and neural network levels.

Similar to the GoF/LoF concept at the molecular level, altered E/I balance at the neuronal circuitry level is the leading hypothesis to explain epilepsy and ASD. In DS, the loss of inhibitory function is expected to increase the E/I ratio, thus triggering epileptic seizures [19]. Likewise, for *SCN2A/SCN8A* GoF mutations, increased excitatory signals are suggested to increase the E/I ratio that contributes to seizure onset [10,25,45]. However, a simple E/I paradigm has less predictive power for explaining GoF *SCN1A* mutations, which cause a spectrum of early onset DEE, as well as less severe phenotypes [88]. Nor does the E/I model explain how LoF *SCN2A/SCN8A* result in autism phenotypes with and without epilepsy [25,43]. Moreover, the relationship between increased E/I ratio and neuronal hyperexcitability, a key phenotype related to autism and epilepsy, is still elusive.

Based on this GoF/LoF paradigm, Scn2a deficiency is expected to impair neuronal excitability. Surprisingly, recent work on *Scn2a* deficiency in *Scn2a*$^{gt/gt}$ mice has revealed a counterintuitive increased E/I ratio and neuronal hyperexcitability [26]. This neuronal hyperexcitability is evident in many cell types of the *Scn2a*$^{gt/gt}$ mice, including striatal MSNs [26,31], pyramidal excitatory neurons [26,64], and hypothalamic neurons [30]. This paradoxical hyperexcitability was similarly observed in the pyramidal neurons of conditional *Scn2a* KO mice [27]. Transcriptomics studies suggest that compensatory reductions in voltage-gated potassium channel expression may explain this unexpected hyperexcitability, as potassium channels are major mediators setting neuronal excitability [26]. This hyperexcitability occurred alongside reduced AP amplitudes and increased E/I ratio. Intriguingly, this increased E/I ratio is achieved by reduced excitatory postsynaptic currents and an even stronger reduction of inhibitory postsynaptic current (reduced IPSCs and EPSCs), resulting in a net increase in E/I ratio [26,31]. Furthermore, the neuronal hyperexcitability in *Scn2a*$^{gt/gt}$ mice was shown to lead to elevated background neuronal activity [31]. This increased basal neuronal activity may serve as 'noise', reducing the network 'signal-to-noise' ratio necessary for typical behaviors (e.g., social interactions) in the *Scn2a*$^{gt/gt}$ mice [31].

humanized gene knock-in is required for translation of gene editors that target human sequences.

Dravet syndrome (DS) is a DEE that is most commonly associated with LoF mutations in *SCN1A* (Nav1.1). Because *SCN1A* is expressed predominantly in GABAergic interneurons [18,19], LoF mutations result in loss of inhibitory neurotransmission and disrupted E/I balance. Many *Scn1a* genetic mouse models have been developed that recapitulate aspects of epileptic phenotypes found in patients with DS, and display spontaneous seizures and sudden unexpected death in epilepsy (SUDEP). These include *Scn1a*$^{+/-}$ knockouts [19] as well as knock-in missense and protein-truncating variants (PTV) identified in humans [20,21]. Most notably, DS mice exhibit reductions in *Scn1a* expression in inhibitory interneurons, decreased interneuron excitability, and reduced inhibitory postsynaptic currents and GABA neurotransmitter release, as well as morphological changes and transcriptional perturbations [9,16,19,22]. Larger animal models have also been developed, including DS rat models [23,24]. Cardiac arrhythmias that are implicated in DS-related SUDEP are currently being studied using rabbit DS models which exhibit more human-like heart rhythms [23].

Different patterns of dysfunction occur as a result of *SCN2A mutations*. *SCN2A* (Nav1.2) is primarily expressed in principal neurons, including excitatory glutamatergic neurons and striatal medium spiny neurons (MSNs) [10,25–27]. Mutations in *SCN2A* are the leading monogenic cause of profound ASD and are strongly linked to epilepsies [25]. Heterozygous knockout *Scn2a*$^{+/-}$ mice only display mild behavioral impairments (unlike *Scn1a*$^{+/-}$ mice), despite the profound ASD and epileptic phenotypes found in humans carrying a heterozygous LoF mutation [6,25]. Homozygous *Scn2a*$^{-/-}$ mice exhibit perinatal lethality [28]. To overcome these limitations, an *Scn2a* gene-trap (*Scn2a*$^{gt/gt}$) model was developed in which *Scn2a* expression was globally reduced to ~20–30% of normal (WT) levels. Importantly, these mice display strong social deficits, circadian rhythm disruption, and other impaired innate behaviors [29–31]. The gene-trap cassette design also allows Nav1.2 levels to be restored by virus-based recombinases and enables restoration of *Scn2a* expression to be studied across the lifespan [29]. In addition, a mouse model with a *Scn2a* p.T1898NfsX27 (Δ1898/+) frameshift mutation has been reported. This frameshift mutation (located at the C terminus) is hypomorphic, leads to a reduced Nav1.2 expression (40–50% of WT), and results in decreased excitability and AP amplitude in pyramidal neurons, as well as increased sociability [32]. Other mouse studies have used knock-in of LoF *SCN2A* mutations found in children with ASD that exhibit social and communication abnormalities, such as *SCN2A*-R102Q from a patient without epilepsy [33]. More recently, there is interest in utilizing *Scn2a*$^{+/-}$ rat models for studying *SCN2A*-related ASD, since rats engage in more sophisticated social behavior [34].

On the other hand, *Scn2a* knock-in murine models were developed to study mixed-function, LoF, and GoF *SCN2A* mutations (Table 1) [35,36]. Global gene knock-in mice carrying the GoF *SCN2A*-p.R1882Q variant exhibit 100% mortality by postnatal day 30 (P30), which reflects SUDEP observed in many patients with GoF *SCN2A* mutations [36]. A mixed-function *SCN2A*-K1422E mutation was identified in a child with developmental delay, infantile spasms, and ASD. This *SCN2A* variant exhibits reduced sodium current density but increased Ca^{2+} conductance, which resulted in reduced AP firing and mixed ion reversal potential; the animal exhibited altered social behavior phenotypes and a rare localized seizure, but interestingly displayed reduced seizure susceptibility [37]. Finally, a LoF *SCN2A*-R853Q variant mouse model has been developed. Although the patients had a severe late-onset seizure phenotype, the heterozygous variant mice displayed no discernible seizure phenotype [38]. Together, these studies demonstrate the complexity of recapitulating human VGSC-related phenotypes in rodent models.

SCN3A (Nav1.3) is predominantly expressed prenatally, and mutations in this gene generally result in DEE [10]. A *Scn3a* gene-trap model has been developed that carries a hypomorphic allele with reduced Nav1.3 function. The heterozygous *Scn3a*$^{+/hyp}$ mice exhibit increased seizure susceptibility, but no spontaneous seizures [39]. Ferret models such as animals expressing mutant *SCN3A* present an interesting opportunity to study the influence of these genes on higher-order structural folds of the brain that are not possible in smaller rodents [40]. *SCN3A*-related channelopathies are rarer than other VGSC disorders, possibly due to lower tolerance of mutations because *SCN3A* is expressed prenatally [10]. Early diagnosis and *in utero* intervention could be a possible direction to treat *SCN3A*-related disorders.

SCN8A (Nav1.6) is more widely expressed in the nervous system, both in excitatory and inhibitory neurons of the central and peripheral nervous systems. *SCN8A* expression increases after *SCN2A* during the postnatal period and across development, and is mainly localized in the distal aspect of the axon initial segment (AIS) and the nodes of Ranvier [7,41,42]. *SCN8A* variants follow a similar phenotypic presentation to *SCN2A*, but have a later age of onset. LoF and haploinsufficiency have been associated with intellectual disability and ASD, with and without seizures [43], and GoF mutations in *SCN8A* patients can result in early infantile DEE (EIEE13/DEE13). Interestingly, both LoF and GoF *SCN8A* mutations have been more strongly associated with movement disorders than other VGSCs, probably because Nav1.6 is expressed in peripheral neurons and plays a crucial role at the neuromuscular junction [44–46]. Several murine models for *SCN8A* have been developed to study GoF mutations, including *SCN8A*-N1768D [47,48] and *SCN8A*-R1620L [49]. Conditional knock-in of the GoF *SCN8A*-p.R1872W mutation into excitatory neurons (using *Emx1–cre* mice) led to a strong seizure phenotype and SUDEP at 3 weeks in heterozygous mice [50]. On the other hand, *Scn8a* complete knockout mice (originally *Scn8a*^{MED}) were used as a model for motor end-plate disease (MED) before later work discovered that *Scn8a* knockout was responsible for the MED phenotype [44]. Although *SCN8A* haploinsufficiency causes autism and ataxia disorders in humans, *Scn8a*$^{+/-}$ mice have a mild or no disease phenotype, while homozygous *Scn8a* knockouts exhibit movement disorder, progressive paralysis, and premature death at 2–4 months of age [44]. Interestingly, conditional knockout of *Scn8a* in Purkinje cells (using *L7/Pcp2–cre* mice) was sufficient to cause motor and social deficits, as well as other ASD-related phenotypes [51].

VGSC β-subunits are auxiliary subunits that facilitate the trafficking and structure of VGSC α-subunits and also play a role in neurite outgrowth, axon projection, and cell adhesion/migration [52]. *SCN1B* has the strongest association with generalized epilepsy with febrile seizures plus (GEFS+) and DEE (DEE52) [53–55]. Other β-subunits are less commonly associated with brain disorders, and are reviewed elsewhere [52]. *Scn1b* null mouse models have been established that exhibit generalized spontaneous seizure and ataxia by postnatal week 2, and later SUDEP [53], which recapitulate the DEE52 phenotypes found in humans.

hiPSC-derived models

hiPSC technology enables highly personalized disease modeling and the development of precision medicine. This is crucial because genetic medicines must target human genetic sequences, and these often differ in animals unless they are engineered with humanized sequences. To generate hiPSC models of disease, somatic cells are taken from patients (or reference individuals) and reprogrammed into a pluripotent state using Yamanaka factors [56]. Genome engineering can then be used to generate isogenic controls of patient lines or to insert mutations into reference hiPSC lines (Figure 3) [57,58]. There is notable variability in hiPSC-derived cultures that can arise because of asynchronous maturation and aberrant differentiation into non-neuronal lineages. For consistency and reproducibility, hiPSC models should follow best practices, including

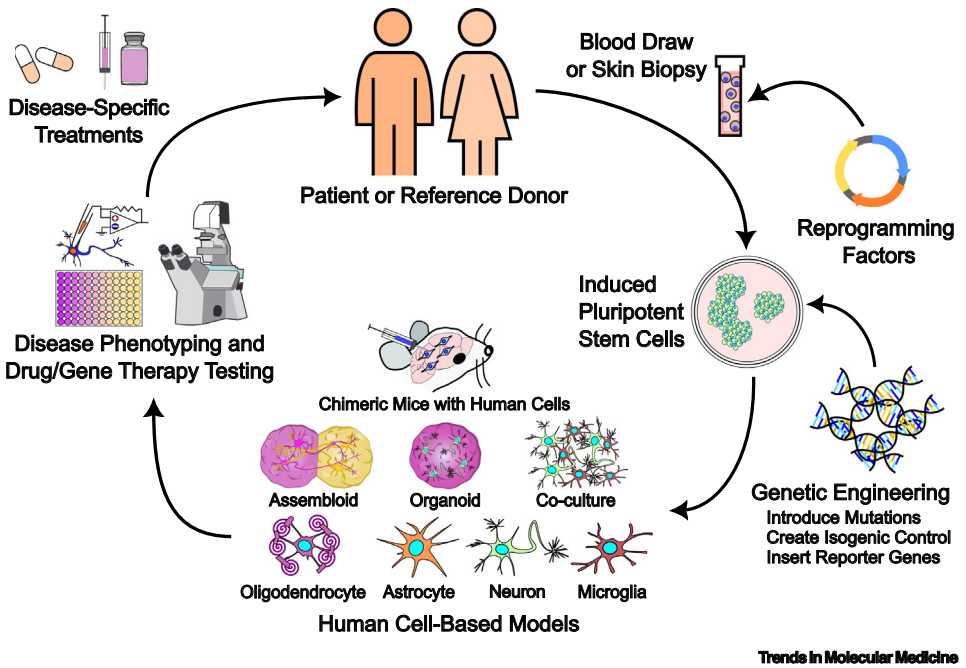

Figure 3. Human induced pluripotent stem cell (hiPSC)-based models for revealing disease phenotypes and advancing the development of next-generation interventions. (Top) From a blood draw or skin biopsy, peripheral mononuclear blood cells, fibroblasts, or other somatic cells can be reprogrammed into a pluripotent state using reprogramming factors such as the Yamanaka factors (OCT4, SOX2, c-MYC, KLF4) to produce hiPSCs. (Right) Additional genetic engineering can be used to generate isogenic controls, insert mutations into reference lines, correction of mutations in patient lines, and the insertion of reporter genes. (Bottom) hiPSCs can be differentiated into various models relevant to studying disease mechanisms of brain disorders, including 2D cultures (such as neurons, astrocytes, oligodendrocytes, and microglia), cocultures, and 3D brain organoids and assembloids. hiPSC-derived cells can also be injected into immunodeficient host mice to generate human xenograft (chimeric) models. (Left) These hiPSC-derived models can then be used to perform disease-specific phenotyping and preclinical testing of drug/gene therapies and to identify and evaluate novel disease-specific precision medicines for further *in vivo* and clinical evaluation in patients.

isogenic controls to account for genetic background differences, the use of multiple clones, assessment of genetic integrity via genomic sequencing or DNA microarrays, regular pluripotency paneling to ensure high-quality hiPSCs, and the use of specific neuronal markers at later stages to validate each differentiation step [59].

hiPSC-derived neuronal models have seen tremendous advance over the 20 years since the discovery of hiPSCs, starting with 2D neuronal [60] and coculture models [61]. Later, brain region-specific organoids that permit 3D structural organization of cells were developed (e.g., cortical, striatal) [62,63]. More recent innovations have expanded these models to include organoid cocultures with microglia and vascularization [64,65], brain-on-a-chip for modeling the BBB [66], and multi-brain region assembloids with and without microglia [59,67,68].

hiPSC-derived brain cultures have been used to study DS-related *SCN1A* haploinsufficiency. The *SCN1A*-S1328P mutation identified in twins with DS was studied in a 2D telencephalic excitatory and medial ganglionic eminence (MGE) inhibitory hiPSC-derived model, where excitatory neurons exhibited normal firing, while MGE-derived inhibitory neurons showed deficits in firing [69]. Furthermore, comparison of different LoF *SCN1A* mutations in hiPSC-derived neurons revealed hyperexcitability and mutation-specific firing patterns which demonstrate that different mutations can have unique effects on neuron function and thus may respond differently to therapeutic interventions [70].

SCN2A-related autism and epilepsy have been studied in 2D hiPSC-derived neurons. Human reference hiPSCs were engineered to study the heterozygous recurring GoF mutation (*SCN2A*-L1342P) found in patients with intractable seizures and DEE [14]. hiPSC-derived cortical neurons carrying this GoF mutation displayed a hyperexcitable phenotype compared to isogenic controls [14,61]. The L1342P neurons exhibited reduced pharmacological responsiveness to an FDA-approved nonspecific sodium channel blocker (phenytoin), but remained sensitive to phrixotoxin-3, an experimental selective Nav1.2 blocker. These results suggest that personalized, mutation-specific drug selection might be important for patients with different VGSC mutations [14]. Other work has used hiPSC-derived neurons from *SCN2A* patients to address how molecular and cellular phenotypes correlate with patient phenotypes [71–73]. Some studies use healthy wild-type lines as a control; however, validation using isogenic controls would help to further distinguish effects related to the genetic background of the hiPSC lines.

hiPSC models of *SCN8A*-related DEE (EIEE13) were used to study GoF *SCN8A* mutations found in children with seizures (e.g., R1872L, V1592L, N1759S) [74]. This genotype–phenotype correlation study revealed that two of the epilepsy patient lines exhibited increased persistent sodium current, while the third had increased resurgent sodium current [74]. Importantly, it was found that persistent and resurgent sodium current GoF mutations exhibited differences in pharmacological responsiveness to phenytoin and riluzole, again suggesting mutation-specific anti-epileptic drug responses.

Isogenic iNeurons (NGN2) – neurons derived from hiPSCs expressing neurogenin 2 (NGN2) to induce neuronal differentiation – have been used to study *SCN3A* DEE variants. The *SCN2A*-I875T variant was shown to produce persistent sodium currents and aberrant firing patterns. It was then shown that the Nav1.3-selective blocker ICA-121431 normalized the excitability of *SCN3A*-I875T mutant neurons [75]. *NGN2*-based iNeuron lines have the advantage of being much faster to generate than dual SMAD inhibition based-patterning but may not accurately mimic neurodevelopmental stages.

Brain organoids and assembloids are another major innovation of hiPSC-derived brain cultures that can provide a more physiologically relevant self-organized 3D environment for neuronal maturation [63]. Multi-omic analysis of cerebral organoid models carrying a *SCN1A* knockout revealed impaired structure, altered neurotransmitter release, and abnormal developmental trajectories [76]. In addition, *SCN2A*-p.C959X variant (found in an individual with ASD) brain organoids cocultured with microglia were shown to have elevated synaptic pruning and synapse elimination [64]. *SCN2A*-variant carrying corticostriatal assembloids and assembloid–microglia coculture models have also been developed which further expand the toolset for uncovering complex disease mechanisms [77,78].

Human cell xenograft models, sometimes called ‘chimeric mice’, represent another advance in disease modeling. This technology combines human cells and rodent models, and leverages the strengths of hiPSC technology to study human cells carrying human genetic sequences within an *in vivo* context. Chimeric human–mouse brain models are established by transplanting progenitor cells into immunodeficient host animals [59,79,80]. Neuronal chimeric mouse models have been developed for several genetically linked neurodevelopmental disorders [81,82], but have not yet been applied to VGSC-related disorders. Another xenograft model involves transplantation of an entire organoid into the rodent brain; this has been performed for cortical organoids carrying a *CACNA1C* epilepsy-associated variant, where the transplant received blood supply, differentiated into multiple cortical neuronal layers, and even responded to environmental stimuli [83].

Precision medicine targeting components of the central dogma

The development of precision medicines for monogenic sodium channelopathies requires careful consideration of the specific VGSC types and mutations. For example, for DS LoF *SCN1A*, therapeutic strategies that increase *SCN1A* expression in inhibitory neurons are being pursued, but these may be contraindicated in GoF *SCN1A* [84–88]. In the case of GoF *SCN2A*/*SCN8A*-related DEE, strategies to inhibit Nav1.2/Nav1.6 expression may be effective [25,36,89,90], whereas in LoF *SCN2A*/*SCN8A* cases it would be beneficial to increase expression [91,92]. Both GoF and LoF mutations in VGSCs can cause disease, and specific interventions are therefore needed for different mutations. Furthermore, effective therapeutics require fine-tuning to determine effective dosing, cell-specific targeting requirements (overexpression of the wrong channel in the wrong cell may be deleterious), and delivery methods. By contrast, gene correction therapy is likely to work on most mutations regardless of the GoF versus LoF properties because the VGSC gene dosage would be regulated through normal homeostatic mechanisms by its natural promoter/enhancer. Over recent years, different strategies targeting various components of the central dogma of molecular biology at the DNA, RNA, and protein levels have been proposed (Figure 4, Key figure) [2,10,12].

Viral vector-based gene replacement/augmentation therapy

Viral vector-based gene replacement/augmentation therapy has entered the clinic, and several adeno-associated virus (AAV)-based therapies have been approved by the FDA and many more are in clinical trials [93]. In the case of CNS disorders, neurotropic AAVs that efficiently cross the BBB are limited, but new brain-specific vectors are under development [94]. Despite this, the AAV9 strain has been approved as a general vector for gene therapy and was recently tested in an *n = 1* trial with hereditary spastic paraplegia [93]. To create an effective viral gene therapy for brain disorders, it is important to consider transgene size, neural tropism, promoters, the developmental window, and BBB delivery.

The use of gene replacement therapy for the delivery of VGSCs is limited by the packaging capacity of AAVs, which can only accommodate ~4.7 kb transgenes [95]. Given that VGSC coding sequences are ~6 kb, alternative strategies are necessary. In the 'dual AAV' strategy, the transgene is split into two parts and packaged separately into two AAVs, where each gene fragment is delivered to the target cells and recombined intracellularly. Several dual AAV recombination strategies have been developed, including DNA- [96], mRNA- [97], and protein-based technologies [98]. Protein-based dual AAV recombination utilizes bacterial inteins which allow the target protein to be reconstituted after each half has been translated with high efficiency [96,99–104]. Intein-mediated, interneuron-specific dual AAV *SCN1A* gene replacement therapy was shown to demonstrate a benefit in preventing SUDEP and seizures in *Scn1a*-deficient mice [102].

A recent study in *Scn1b* null mice demonstrated that AAV vector-mediated restoration of *Scn1b* was able to prolong the mouse lifespan when administered at the neonatal stage, but administration later than P10 was unable to prevent premature death [53]. The use of a single AAV–*Scn1b* was made possible due to the small size of the *SCN1B* gene. This study suggests that earlier intervention is more effective, either because of the role of *Scn1b* in the developmental trajectory or improved biodistribution of the vector within a younger brain. Interestingly, in other cases VGSC-related disorders may be amenable to gene therapy even at the adult stage [31,88,105]. Therefore, the timing of gene therapy could be a crucial factor to consider for the treatment of different VGSC-related disorders.

An alternative to AAV, CAV-2, is an attractive viral vector because it can accommodate a larger cargo payload (~35 kb) and displays neuronal tropism, retrograde transport, low

Key figure

Precision medicine strategies to treat voltage-gated sodium channelopathies

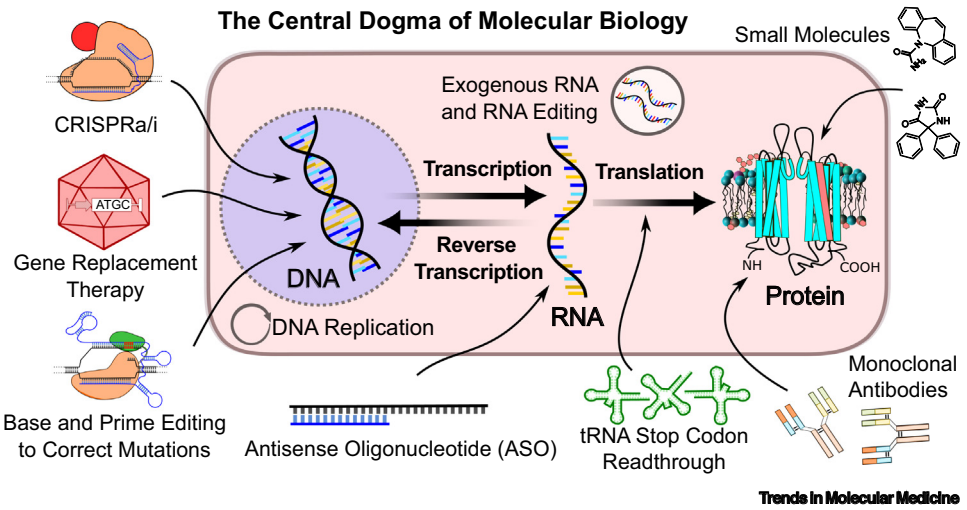

Figure 4. DNA, RNA, and protein are the key components in the central dogma of molecular biology which can be targeted to treat monogenic disorders. Strategies that restore, replace, or mitigate the consequences of pathogenic mutations are under various stages of development. (Right) Traditionally, small molecules that target proteins are the first-line treatment for managing the symptoms caused by voltage-gated sodium channelopathy (VGSC) disorders, including sodium channel blockers (e.g., carbamazepine) and other antiepileptic drugs (e.g., valproic acid). Monoclonal antibodies have revolutionized immunological disease management by targeting and eliminating harmful molecules, and may be able to target sodium channels. (Middle) At the RNA level, antisense oligonucleotides (ASOs) can be used to modulate protein expression, including in an allele-specific manner. RNA editing may also be used to correct mutations at the RNA level without changing the DNA makeup. Finally, engineered tRNAs that can 'readthrough' premature stop codons to produce full-length functional proteins may be promising for nonsense mutations. Exogenous encapsulated RNA can be used to deliver a variety of therapeutics. (Left) At the DNA level, gene replacement therapies are another avenue to treat loss-of-function variants pending the development of effective cell type-specific delivery vehicles. Currently, viral vectors appear to be the most widely studied for gene delivery to the brain. CRISPR activation/interference (CRISPRa/i) are also promising technologies that may have a benefit for gain- and loss-of-function mutations by regulating gene expression at the promoters of VGSC genes. Gene-editing technology has seen major advances in recent years. In particular, CRISPR prime and base editing hold tremendous promise for treating monogenic disease at its root by correcting disease-causing genetic mutations in the genome.

immunogenicity, and long-term expression in the CNS [106]. CAV-2-mediated delivery of *Scn1a* by direct injection into the hippocampus of DS mice reduced seizures and improved survival [107]. High-capacity human adenoviral vectors (HC-AdVs) have also been developed for large transgene delivery into *Scn1a*$^{+/-}$ DS mice, which improved survival and attenuated motor and behavioral deficits [85]. These 'helper-dependent' vectors do not contain all viral genes and thus require additional vectors for their production, but can accommodate transgenes of up to ~35 kb. New AAV capsids that display a human transferrin receptor-binding domain (hTfR) represent an exciting advance and are being explored as vectors for brain delivery via systemic IV administration [94].

CRISPR-based genome and RNA editing

Direct targeting of the fundamental cause of monogenic disorders through gene editing is becoming feasible. CRISPR technology allows precise genetic targeting, and CRISPR-based

editors have rapidly advanced from fundamental biological discovery to FDA approval in an astounding 11 years [108].

While traditional CRISPR-based gene editing is a tremendous tool for genetic engineering, it relies on double-stranded DNA breaks (DSB). Thus, its therapeutic potential suffers from possible off-target and on-target damage including insertions and deletions (indels) caused by non-homologous and microhomology-mediated end-joining (NHEJ/MMEJ). Thus, traditional CRISPR is not ideal for correcting mutations [89] but is useful for targeted gene disruption or knockdown by introducing indels. In a preclinical setting, CRISPR/Cas9 was used to knock out the GoF *Scn8a*-N1768D variant in a mouse model engineered with multiple silent mutations; knockout led to improved survival and seizure reduction [109]. Base editing (BE), developed in 2016, uses a modified Cas9–deaminase fusion protein that creates only a single-stranded DNA nick (a 'nickase'), which limits the risk of inducing DSBs [110]. The BE-deaminase domains are limited to four of 12 base substitutions – C>T, A>G, T>C and G>A. Notably, BE can have unintended bystander edits in which conversion of the wrong nucleotide may occur within the editing window. That said, BE demonstrated utility in rescuing seizures and SUDEP in an *SCN8A*-R1872W GoF mouse model [104]. More recently, prime editing (PE) has been developed. PE contains an engineered reverse transcriptase fused with a Cas9 nickase, which works with the PE guide RNA (pegRNA) to reverse transcribe desired edits into the genome [111]. PE is capable of correcting single base pairs and making small insertions and deletions, and has a lower risk of unintended DNA damage (e.g., indels) [111,112]. Recently, editing efficiencies of >95% have been achieved in hiPSCs [113]. The choice of traditional CRISPR versus PE or BE depends on the exact nature of the required sequence alteration. While a streamlined 'mutation-agnostic' editing platform is desirable, the editing efficiency of different mutations is currently variable and needs to be experimentally determined.

Because CRISPR-based editing systems are large, delivery to the brain is a major challenge. While there are ongoing efforts to reduce the size of CRISPR-based editors by using hypercompact Cas9 ancestry proteins that can fit into a single AAV [114], dual AAV is still the most viable delivery approach for brain disorders at this time. Prime and base editors have been successfully delivered to the brain using the dual AAV intein-based systems and have achieved successful editing [99,104,115]. However, virus-based gene-editing vectors, which can persist for long periods of time, may not be ideal as they could continuously interact with DNA and potentially increase the risk of off-target effects. This may be circumvented if self-limiting editor designs can be developed. Recently, CRISPR editors have been delivered as ribonucleoproteins (RNPs) using engineered virus-like particles (eVLPs) and extracellular vesicles (EVs), and have demonstrated successful *in vivo* genome editing [116–119]. The cells located near the injection site were found to have a higher editing efficiency, likely due to limited diffusion of eVLPs through the brain [117]. Lipid nanoparticle delivery has also been used for CRISPR/Cas9 delivery via intracerebral injection and has achieved promising results [120]. While nonviral vectors do not persist in cells for extended periods of time, the narrow perivascular space reduces their biodistribution throughout the whole brain. Thus, future optimization will be essential to achieve effective editing of the whole brain for the treatment of monogenic brain disorders.

RNA editing is a technology that can correct mutations at the mRNA level and does not alter genomic DNA. One RNA-editing approach uses adenosine deaminase acting on RNA type 2 (ADAR2) which converts adenosine through a two-step process to guanosine (A>G edit). This technology utilizes a guide RNA to target specific bases and has been used to edit the RNA sequence of voltage-gated calcium [121] and potassium channels [122]. In addition to ADAR

Clinician's corner

In children with developmental and epileptic encephalopathy, genetic testing is essential to determine the specific gene and genotype to accurately inform the correct selection of anti-epileptic drugs.

Patients with Dravet syndrome often do not respond well to sodium channel blockers, and valproic acid and the new stiripentol are considered as first-line treatments.

Fenfluramine is a new potential serotonergic add-on that has been approved by the FDA for seizure control in DS.

Patients with *SCN1A* GoF mutations are generally responsive to sodium channel blockers.

In cases of *SCN2A*-related disorders, many patients who have clear *SCN2A* GoF DEE with early-onset seizures are expected to respond well to sodium channel blockers such as carbamazepine and phenytoin.

Sodium channel blockers may be less well tolerated in LoF *SCN2A* and *SCN8A* patients with late-onset seizures, but are sometimes used for status epilepticus.

It is suspected that individuals with neurodevelopmental disorders, including profound ASD, may have an underlying genetic basis for their condition. However, because treatment options are limited for ASD, genetic testing is not widely adopted in these patients. If the genetic variants responsible cannot be identified, genetically defined subpopulations of ASD cannot be used to advance basic science and drug development (a 'catch-22').

It is important to build a consensus and demonstrate the benefits of genetic testing of children with ASD (and other neurodevelopmental disorders) to stakeholders, which would help to advance precision medicine for this population.

Precision medicine, by definition, is precise and targets a specific or small population. Accordingly, there is a limited pool of patients who are eligible for clinical trials. For example, gene

editing, CRISPR systems have been developed that edit RNA sequences. Cas13 is a family of CRISPR-associated enzyme that can cleave RNA. Researchers have generated RNA editors that use enzymatically deactivated dCas13 fused to an ADAR domain to enable specific A>G [123], and C>U conversions [124]. While these technologies have not been used for VGSCs, they may allow treatment of a variety of diseases without the need to modify DNA.

Modulation of gene and protein expression

Modulation of gene and protein expression is another strategy that has been shown to be effective in disease models of various sodium channelopathies. However, the design of gene modulation therapies for VGSC variants needs to be carefully considered to prevent unintended side effects related to overexpression of the pathogenic allele. Allele-specific approaches have been proposed that only target the pathogenic variant. Allele specificity is likely to be more accurate for small insertions and deletions [125] than for missense variants because a single base-pair difference may not achieve sufficient allele specificity. To improve variant targeting, single-nucleotide polymorphisms (SNPs) identified on the pathogenic allele through long-read sequencing may be useful [126]. The SNP-based allele-specific strategy may also allow the development of 'mutation-agnostic' interventions for treating a broader patient population.

Recent successes in gene modulation technologies have been achieved with antisense oligonucleotides (ASOs) – short single-stranded oligonucleotides that alter RNA structure, splicing, and degradation, and thus modulate protein expression [2]. In *SCN2A*-p.R1882Q GoF variant mice with 100% mortality, gapmer ASO-mediated knockdown extended mutant mouse lifespan and reduced the frequency of seizures [36]. ASOs are also currently in clinical trials for *SCN2A* GoF mutations (PRAX-222, NCT05737784) [127]. A personalized allele-specific ASO knockdown is being tested in an $n = 1$ trial for a child carrying a GoF *SCN2A* mutation (R853Q) (nL-SCN2A-002, NCT06314490). In addition, ASOs have been applied successfully in mouse models of *SCN8A*-related epilepsy and were able to alleviate seizure-related phenotypes [89]. ASOs can also be designed to increase protein expression and have been used for upregulation of Nav1.1 in DS mouse models where they reduced seizures and SUDEP by using 'targeted augmentation of nuclear gene output' (TANGO) [128]. This TANGO ASO targets a 'poison exon' of *SCN1A* pre-mRNA, and consequently increases mRNA and protein levels and upregulates global Nav1.1 levels which can compensate for haploinsufficiency [84,128]. This TANGO ASO strategy (zorevunersen, Stokes Therapeutics; NCT04740476) for *SCN1A* is in clinical trials. Importantly, the TANGO strategy is contraindicated by *SCN1A* GoF variants because increased expression of the mutant allele may have detrimental effects in these patients.

CRISPRa/i use catalytically inactive CRISPR–dCas fusion proteins coupled to either a transcriptional activator (e.g., VP64) for CRISPRa or a transcriptional repressor (e.g., KRAB) for CRISPRi, and can thereby modulate target gene expression. In *Scn2a*$^{+/-}$ haploinsufficient mice, CRISPRa was shown to increase *Scn2a* expression and rescue cellular phenotypes [91,92]. This approach appears to be well tolerated and did not result in pathogenic consequences as a result of overexpression. Similar CRISPRa strategies have been used to restore inhibitory interneuron excitability by targeting *Scn1a* promoters in DS mice, and led to a decrease in seizure behavior [86]. In contrast to CRISPRa strategies, CRISPRi could be useful in some instances to target and reduce pathogenic gene expression [129]; however, silencing both the healthy and pathogenic alleles may be deleterious.

Delivery of engineered transcription factors (eTFs) to modulate gene expression is also being attempted. In a DS mouse model, treatment with AAV9-RE^{GABA}-eTF^{SC1A} to target the promoter of *Scn1a* with an eTF in GABAergic interneurons increases Nav1.1 expression and alleviates

replacement therapies may be broadly applicable to haploinsufficiency mutations (n = 10s to 100s), but a gene-editing therapy for a specific mutation would necessitate an $n = 1$ trial.

Platform-based approval of gene editing would help to increase patient access to these treatments and lower the costs for expanded trials. The overall development cost of precision therapies for the first few patients will be high (~$10–100 million), which requires support from the government and generous philanthropic partners. It is hopeful that genetic medicines may follow the same trend as genome sequencing, where the first human genome cost >$3 billion but can now be sequenced for <$3000 per genome. Therefore, we expect gene therapy will become accessible to an ever-expanding population and offer long-term savings and better health outcomes for society at large.

seizures [87]. This strategy, like many other gene modulatory modalities, requires the use of cell type-specific promoters since TFs can regulate broad-scale signal transduction pathways.

Targeting translational machinery to modify gene expression has been gaining interest. Nonsense mutations are fairly common, and ~11% of single-base substitutions result in a premature stop codon [130]. Typically, nonsense mutations that occur early in the mRNA transcripts undergo nonsense-mediated decay which results in haploinsufficiency. To target these mutations, a translational readthrough strategy has been developed where suppressor tRNAs (sup-tRNAs) target ribosomal machinery to read through stop codons. These sup-tRNAs have been delivered using AAV-based gene therapy to rescue nonsense mutations [130]. This technology, in theory, should not have the potential side effects of pathological overexpression and could limit target gene expression to the appropriate cell type. However, strategies will be necessary to maximally spare natural stop codon readthrough to further advance clinical translation.

Small molecules and antibodies to target proteins can also be used in a precision medicine manner, including transcriptomic-based drug discovery [30,131]. An expanding array of small molecules have been developed to target VGSCs and treat DEE, but fewer pharmacological treatment options are available for ASD. These have been discussed in other reviews [4,10,12,25,105] and will not be covered here.

Concluding remarks

Research is advancing the treatment landscape for VSCG-related epilepsy and autism, though many questions remain to be answered (see Outstanding questions). Unlike traditional approaches, precision medicine customizes interventions based on the unique genetic mutation of each individual, leading to more effective treatments. Animal models of VGSC-related epilepsy and ASD have revealed phenotypes that provide valuable insights into the mechanisms of disease and provide a platform for evaluating precision medicine. Furthermore, advances in hiPSC-derived models offer a powerful platform for studying human disease mechanisms and testing genetic interventions targeting human sequences. Gene replacement therapies and recent advances in CRISPR, BE, and PE also offer a promising toolkit for intervention. Technologies that enable precise modulation of gene and protein expression are closer to clinical realization, including ASOs, CRISPRa/i, eTFs, and tRNAs.

Despite these recent trends in therapeutic development for VGSC-related epilepsy and autism, there are significant regulatory hurdles in bringing new precision medicines to the market for patients with rare diseases. To overcome these systematic barriers, families, patient advocacy groups, clinicians, and researchers should come together to implement a comprehensive research-readiness infrastructure to facilitate clinical trial design and accelerate FDA approval [132–134]. Working together with all the stakeholders to advance research and beyond, a precision medicine future for monogenic brain disorders will undoubtedly become a reality.

Outstanding questions

Do animal and human iPSC models have sufficient predictive power to evaluate the safety and efficacy profile for translating gene therapies into human clinical studies?

There is a move away from animal models for translational research; therefore, how can human cell-based models be improved to more accurately model human diseases and enable clinical translation without the use of animals?

The effective delivery of larger biological therapeutics, including gene therapies to the brain, remains limited: what are the suitable delivery methods for different gene therapies to produce benefit for patients?

What strategies (e.g., cell type-specific promoters) should be used to achieve selective expression of the transgene in the target cell type?

What percentage of neurons needs to be targeted to have a clinically measurable improvement?

Because many VGSCs are important for neurodevelopment, what is the appropriate timing to administer gene therapy? Is there a point where the net benefit is too low?

How durable do gene replacement therapies need to be? Would there be a need for repeated dosing and to what extent can immunosuppression permit re-dosing?

Given the high costs of development and the small patient populations, how can gene therapy clinical trials, including $n = 1$ trials, be designed to ensure economic feasibility, sustainability, and equitable access?

What levels of cost and risk–benefit will patients/families, clinicians, scientists, and regulators consider appropriate for precision medicine interventions, particularly in conditions that are not life-threatening but cause profound disability?

Acknowledgments

This work of the Yang laboratory was supported by the National Institutes of Health (NIH) National Institute of Neurological Disorders and Stroke (NINDS) grants R01 NS117585 and R01 NS123154 (to Y.Y.). M.R. is supported by the Lillian Gilbreth Engineering Postdoctoral Fellowship from Purdue University. The Yang laboratory thanks the FamilieSCN2A Foundation for the Action Potential and the Hodgkin–Huxley Research Awards.

Declaration of interests

The authors declare no conflicts of interest.

References

1. Musunuru, K. *et al.* (2025) Patient-specific in vivo gene editing to treat a rare genetic disease. *N. Engl. J. Med.* 392, 22
2. Devinsky, O. *et al.* (2025) Gene therapies for neurogenetic disorders. *Trends Mol. Med.* 31, 814–826
3. López-Rivera, J.A. *et al.* (2020) A catalogue of new incidence estimates of monogenic neurodevelopmental disorders caused by de novo variants. *Brain* 143, 1099–1105
4. Knowles, J.K. *et al.* (2022) Precision medicine for genetic epilepsy on the horizon: recent advances, present challenges, and suggestions for continued progress. *Epilepsia* 63, 2461–2475
5. Lyu, H. *et al.* (2023) Clinical and electrophysiological features of SCN8A variants causing episodic or chronic ataxia. *eBioMedicine* 98, 104855
6. Berg, A.T. *et al.* (2024) Expanded clinical phenotype spectrum correlates with variant function in SCN2A-related disorders. *Brain* 147, 2761–2774
7. Hu, W. *et al.* (2009) Distinct contributions of Nav1.6 and Nav1.2 in action potential initiation and backpropagation. *Nat. Neurosci.* 12, 996–1002
8. Spratt, P.W.E. *et al.* (2019) The autism-associated gene Scn2a contributes to dendritic excitability and synaptic function in the prefrontal cortex. *Neuron* 103, 673–685
9. Mantegazza, M. *et al.* (2021) Sodium channelopathies of skeletal muscle and brain. *Physiol. Rev.* 101, 1633–1689
10. Brunklaus, A. and Lal, D. (2020) Sodium channel epilepsies and neurodevelopmental disorders: from disease mechanisms to clinical application. *Dev. Med. Child Neurol.* 62, 784–792
11. Vanoye, C.G. *et al.* (2024) Molecular and cellular context influences SCN8A variant function. *JCI Insight* 9, e177530
12. Meisler, M.H. *et al.* (2021) Sodium channelopathies in neurodevelopmental disorders. *Nat. Rev. Neurosci.* 22, 152–166
13. Ben-Shalom, R. *et al.* (2017) Opposing effects on NaV1.2 function underlie differences between SCN2A variants observed in individuals with autism spectrum disorder or infantile seizures. *Biol. Psychiatry* 82, 224–232
14. Que, Z. *et al.* (2021) Hyperexcitability and pharmacological responsiveness of cortical neurons derived from human iPSCs carrying epilepsy-associated sodium channel Nav1.2-L1342P genetic variant. *J. Neurosci.* 41, 10194–10208
15. Doorn, N. *et al.* (2023) An in silico and in vitro human neuronal network model reveals cellular mechanisms beyond NaV1.1 underlying Dravet syndrome. *Stem Cell Rep.* 18, 1686–1700
16. Mistry, A.M. *et al.* (2014) Strain- and age-dependent hippocampal neuron sodium currents correlate with epilepsy severity in Dravet syndrome mice. *Neurobiol. Dis.* 65, 1–11
17. Zhang, Z. *et al.* (2025) Gene therapies alleviate absence epilepsy associated with Scn2a deficiency in DBA/2J mice. *bioRxiv* Published online June 6, 2025. https://doi.org/10.1101/2025.06.03.657652
18. Ogiwara, I. *et al.* (2007) Nav1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. *J. Neurosci.* 27, 5903–5914
19. Yu, F.H. *et al.* (2006) Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. *Nat. Neurosci.* 9, 1142–1149
20. Martin, M.S. *et al.* (2010) Altered function of the SCN1A voltage-gated sodium channel leads to γ-aminobutyric acid-ergic (GABAergic) interneuron abnormalities. *J. Biol. Chem.* 285, 9823–9834
21. Das, A. *et al.* (2021) Interneuron dysfunction in a new mouse model of SCN1A GEFS+. *eNeuro* 8 ENEURO.0394-20.2021
22. Ricobaraza, A. *et al.* (2019) Epilepsy and neuropsychiatric comorbidities in mice carrying a recurrent Dravet syndrome SCN1A missense mutation. *Sci. Rep.* 9, 14172
23. Ramos-Mondragon, R. *et al.* (2022) Development of a transgenic rabbit model of Dravet syndrome. *Circulation* 146, A12541
24. Li, M. *et al.* (2023) A novel rat model of Dravet syndrome recapitulates clinical hallmarks. *Neurobiol. Dis.* 184, 106193
25. Sanders, S.J. *et al.* (2018) Progress in understanding and treating SCN2A-*Mediated Disorders*. *Trends Neurosci.* 41, 442–456
26. Zhang, J. *et al.* (2021) Severe deficiency of the voltage-gated sodium channel NaV1.2 elevates neuronal excitability in adult mice. *Cell Rep.* 36, 109495
27. Spratt, P.W.E. *et al.* (2021) Paradoxical hyperexcitability from NaV1.2 sodium channel loss in neocortical pyramidal cells. *Cell Rep.* 36, 109483
28. Planells-Cases, R. *et al.* (2000) Neuronal death and perinatal lethality in voltage-gated sodium channel αii-deficient mice. *Biophys. J.* 78, 2878–2891
29. Eaton, M. *et al.* (2021) Generation and basic characterization of a gene-trap knockout mouse model of Scn2a with a substantial reduction of voltage-gated sodium channel Nav1.2 expression. *Genes Brain Behav.* 20, e12725
30. Ma, Z. *et al.* (2022) Deficiency of autism-related Scn2a gene in mice disrupts sleep patterns and circadian rhythms. *Neurobiol. Dis.* 168, 105690
31. Zhang, J. *et al.* (2025) Restoration of excitation/inhibition balance enhances neuronal signal-to-noise ratio and rescues social deficits in autism-associated Scn2a-deficiency. *bioRxiv* Published online March 5, 2025. https://doi.org/10.1101/2025.03.04.641498
32. Wang, H.-G. *et al.* (2021) Scn2a severe hypomorphic mutation decreases excitatory synaptic input and causes autism-associated behaviors. *JCI Insight* 6, e150698
33. Gao, Y. *et al.* (2024) Proximity analysis of native proteomes reveals phenotypic modifiers in a mouse model of autism and related neurodevelopmental conditions. *Nat. Commun.* 15, 6801
34. Klibaite, U. *et al.* (2025) Mapping the landscape of social behavior. *Cell* 188, 2249–2266
35. Kearney, J.A. *et al.* (2001) A gain-of-function mutation in the sodium channel gene Scn2a results in seizures and behavioral abnormalities. *Neuroscience* 102, 307–317
36. Li, M. *et al.* (2021) Antisense oligonucleotide therapy reduces seizures and extends life span in an SCN2A gain-of-function epilepsy model. *J. Clin. Invest.* 131, e152079
37. Echevarria-Cooper, D.M. *et al.* (2022) Cellular and behavioral effects of altered NaV1.2 sodium channel ion permeability in Scn2aK1422E mice. *Hum. Mol. Genet.* 31, 2964–2988
38. Jia, L. *et al.* (2024) Variant-specific in vitro neuronal network phenotypes and drug sensitivity in SCN2A developmental and epileptic encephalopathy. *J. Neurochem.* 168, 3950–3961
39. Lamar, T. *et al.* (2017) SCN3A deficiency associated with increased seizure susceptibility. *Neurobiol. Dis.* 102, 38–48
40. Smith, R.S. *et al.* (2018) Sodium channel SCN3A (NaV1.3) regulation of human cerebral cortical folding and oral motor development. *Neuron* 99, 905–913
41. Caldwell, J.H. *et al.* (2000) Sodium channel Nav1.6 is localized at nodes of Ranvier, dendrites, and synapses. *Proc. Natl. Acad. Sci.* 97, 5616–5620
42. Ye, M. *et al.* (2018) Differential roles of NaV1.2 and NaV1.6 in regulating neuronal excitability at febrile temperature and distinct contributions to febrile seizures. *Sci. Rep.* 8, 753
43. Wagnon, J.L. *et al.* (2017) Loss-of-function variants of SCN8A in intellectual disability without seizures. *Neurol. Genet.* 3, e170
44. Burgess, D.L. *et al.* (1995) Mutation of a new sodium channel gene, Scn8a, in the mouse mutant "motor endplate disease". *Nat. Genet.* 10, 461–465
45. Meisler, M.H. (2019) SCN8A encephalopathy: mechanisms and models. *Epilepsia* 60, S86–S91
46. Duchen, L.W. (1970) Hereditary motor end-plate disease in the mouse: light and electron microscopic studies. *J. Neurol. Neurosurg. Psychiatry* 33, 238–250
47. Lopez-Santiago, L.F. *et al.* (2017) Neuronal hyperexcitability in a mouse model of SCN8A epileptic encephalopathy. *Proc. Natl. Acad. Sci.* 114, 2383–2388
48. Yu, W. *et al.* (2024) Dentate gyrus granule cells are a locus of pathology in Scn8a developmental encephalopathy. *Neurobiol. Dis.* 199, 106591
49. Wong, J.C. *et al.* (2021) Autistic-like behavior, spontaneous seizures, and increased neuronal excitability in a Scn8a mouse model. *Neuropsychopharmacology* 46, 2011–2020

50. Bunton-Stasyshyn, R.K.A. *et al.* (2019) Prominent role of forebrain excitatory neurons in SCN8A encephalopathy. *Brain* 142, 362–375
51. Yang, X. *et al.* (2022) Social deficits and cerebellar degeneration in Purkinje cell Scn8a knockout mice. *Front. Mol. Neurosci.* 15, 822129
52. Bouza, A.A. and Isom, L.L. (2018) Voltage-gated sodium channel β subunits and their related diseases. *Handb. Exp. Pharmacol.* 246, 423–450
53. Chen, C. *et al.* (2025) Neonatal but not juvenile gene therapy reduces seizures and prolongs lifespan in SCN1B-Dravet syndrome mice. *J. Clin. Invest.* 135, e182584
54. Zhu, Z. *et al.* (2022) SCN1B genetic variants: a review of the spectrum of clinical phenotypes and a report of early myoclonic encephalopathy. *Child. Basel Switz.* 9, 1507
55. Chen, C. *et al.* (2004) Mice lacking sodium channel β1 subunits display defects in neuronal excitability, sodium channel expression, and nodal architecture. *J. Neurosci.* 24, 4030–4042
56. Takahashi, K. and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663–676
57. Pantazis, C.B. *et al.* (2022) A reference human induced pluripotent stem cell line for large-scale collaborative studies. *Cell Stem Cell* 29, 1685–1702.e22
58. Skarnes, W.C. *et al.* (2019) Improving homology-directed repair efficiency in human stem cells. *Methods San Diego Calif.* 164–165, 18–28
59. Pa ca, S.P. *et al.* (2024) A framework for neural organoids, assembloids and transplantation studies. *Nature* 639, 315–320
60. Okano, H. and Yamanaka, S. (2014) iPS cell technologies: significance and applications to CNS regeneration and disease. *Mol. Brain* 7, 22
61. Que, Z. *et al.* (2025) Human iPSC-derived microglia sense and dampen hyperexcitability of cortical neurons carrying the epilepsy-associated SCN2A-L1342P mutation. *J. Neurosci.* 45, e2027232024
62. Urresti, J. *et al.* (2021) Cortical organoids model early brain development disrupted by 16p11.2 copy number variants in autism. *Mol. Psychiatry* 26, 7560–7580
63. Olivero-Acosta, M.I. *et al.* (2025) Epilepsy-associated SCN2A-L1342P mutation drives network hyperexcitability and widespread transcriptomic changes in human cortical organoids. *bioRxiv* Published online August 19, 2025. https://doi.org/10.1101/2025.08.18.670956
64. Wu, J. *et al.* (2024) Microglial over-pruning of synapses during development in autism-associated SCN2A-deficient mice and human cerebral organoids. *Mol. Psychiatry* 29, 2424–2437
65. Cakir, B. *et al.* (2019) Engineering of human brain organoids with a functional vascular-like system. *Nat. Methods* 16, 1169–1175
66. Servais, B. *et al.* (2024) Engineering brain-on-a-chip platforms. *Nat. Rev. Bioeng.* 2, 691–709
67. Kim, J. *et al.* (2024) Human assembloids reveal the consequences of CACNA1G gene variants in the thalamocortical pathway. *Neuron* 112, 4048–4059.e7
68. Patton, M.H. *et al.* (2024) Synaptic plasticity in human thalamocortical assembloids. *Cell Rep.* 43, 114503
69. Sun, Y. *et al.* (2016) A deleterious Nav1.1 mutation selectively impairs telencephalic inhibitory neurons derived from Dravet syndrome patients. *eLife* 5, e13073
70. van Hugte, E.J.H. *et al.* (2023) SCN1A-deficient excitatory neuronal networks display mutation-specific phenotypes. *Brain* 146, 5153–5167
71. Asadollahi, R. *et al.* (2023) Pathogenic SCN2A variants cause early-stage dysfunction in patient-derived neurons. *Hum. Mol. Genet.* 32, 2192–2204
72. Mao, M. *et al.* (2024) Distinctive *in vitro* phenotypes in iPSC-derived neurons from patients with gain- and loss-of-function SCN2A developmental and epileptic encephalopathy. *J. Neurosci.* 44, e0692232023
73. Brown, C.O. *et al.* (2024) Disruption of the autism-associated gene SCN2A alters synaptic development and neuronal signaling in patient iPSC-glutamatergic neurons. *Front. Cell. Neurosci.* 17, 1239069
74. Tidball, A.M. *et al.* (2020) Variant-specific changes in persistent or resurgent sodium current in SCN8A-related epilepsy patient-derived neurons. *Brain* 143, 3025–3040
75. Qu, G. *et al.* (2024) Targeted blockade of aberrant sodium current in a stem cell-derived neuron model of SCN3A encephalopathy. *Brain* 147, 1247–1263
76. Koh, B. *et al.* (2024) Unraveling the molecular landscape of SCN1A gene knockout in cerebral organoids: a multiomics approach utilizing proteomics, lipidomics, and transcriptomics. *ACS Omega* 9, 39804–39816
77. Wu, J. *et al.* (2025) Human microglia in brain assembloids display region-specific diversity and respond to hyperexcitable neurons carrying SCN2A mutation: microglial diversity and response in assembloidsbio. *bioRxiv* Published online June 4, 2025. https://doi.org/10.1101/2025.06.04.657874
78. Chen, X. *et al.* (2025) Autism-associated SCN2A deficiency disrupts cortico-striatal circuitry in human brain assembloids. *bioRxiv* Published online June 3, 2025. https://doi.org/10.1101/2025.06.02.657036
79. Xu, R. *et al.* (2020) Human iPSC-derived mature microglia retain their identity and functionally integrate in the chimeric mouse brain. *Nat. Commun.* 11, 1577
80. Xu, R. *et al.* (2019) OLIG2 drives abnormal neurodevelopmental phenotypes in human iPSC-based organoid and chimeric mouse models of Down syndrome. *Cell Stem Cell* 24, 908–926.e8
81. Vitrac, A. *et al.* (2020) A chimeric mouse model to study human iPSC-derived neurons: the case of a truncating SHANK3 mutation. *Sci. Rep.* 10, 13315
82. Nadadhur, A.G. *et al.* (2019) Neuron–glia interactions increase neuronal phenotypes in tuberous sclerosis complex patient iPSC-derived models. *Stem Cell Rep.* 12, 42–56
83. Revah, O. *et al.* (2022) Maturation and circuit integration of transplanted human cortical organoids. *Nature* 610, 319–326
84. Han, Z. *et al.* (2020) Antisense oligonucleotides increase Scn1a expression and reduce seizures and SUDEP incidence in a mouse model of Dravet syndrome. *Sci. Transl. Med.* 12, eaaz6100
85. Mora-Jimenez, L. *et al.* (2021) Transfer of SCN1A to the brain of adolescent mouse model of Dravet syndrome improves epileptic, motor, and behavioral manifestations. *Mol. Ther. Nucleic Acids* 25, 585–602
86. Colasante, G. *et al.* (2020) dCas9-based Scn1a gene activation restores inhibitory interneuron excitability and attenuates seizures in Dravet syndrome mice. *Mol. Ther.* 28, 235–253
87. Tanenhaus, A. *et al.* (2022) Cell-selective adeno-associated virus-mediated SCN1A gene regulation therapy rescues mortality and seizure phenotypes in a Dravet syndrome mouse model and is well tolerated in nonhuman primates. *Hum. Gene Ther.* 33, 579–597
88. Brunklaus, A. *et al.* (2022) The gain of function SCN1A disorder spectrum: novel epilepsy phenotypes and therapeutic implications. *Brain* 145, 3816–3831
89. Hill, S.F. *et al.* (2024) Long-term downregulation of the sodium channel gene Scn8a is therapeutic in mouse models of SCN8A epilepsy. *Ann. Neurol.* 95, 754–759
90. Lenk, G.M. *et al.* (2020) Scn8a antisense oligonucleotide is protective in mouse models of SCN8A encephalopathy and Dravet syndrome. *Ann. Neurol.* 87, 339–346
91. Wang, C. *et al.* (2024) Impaired cerebellar plasticity hypersensitizes sensory reflexes in SCN2A-associated ASD. *Neuron* 112, 1444–1455.e5
92. Tamura, S. *et al.* (2025) CRISPR activation for SCN2A-related neurodevelopmental disorders. *Nature* 646, 983–991. https://doi.org/10.1038/s41586-025-09522-w
93. Dowling, J.J. *et al.* (2024) AAV gene therapy for hereditary spastic paraplegia type 50: a phase 1 trial in a single patient. *Nat. Med.* 30, 1882–1887
94. Huang, Q. *et al.* (2024) An AAV capsid reprogrammed to bind human transferrin receptor mediates brain-wide gene delivery. *Science* 384, 1220–1227
95. Zhu, D. *et al.* (2021) Adeno-associated virus vector for central nervous system gene therapy. *Trends Mol. Med.* 27, 524–537
96. Wu, J. *et al.* (2021) Single and dual vector gene therapy with AAV9-PHP.B rescues hearing in Tmc1mutant mice. *Mol. Ther.* 29, 973–988

97. Riedmayr, L.M. *et al.* (2023) mRNA trans-splicing dual AAV vectors for (epi)genome editing and gene therapy. *Nat. Commun.* 14, 6578
98. Tornabene, P. *et al.* (2019) Intein-mediated protein trans-splicing expands adeno-associated virus transfer capacity in the retina. *Sci. Transl. Med.* 11, eaav4523
99. Davis, J.R. *et al.* (2024) Efficient prime editing in mouse brain, liver and heart with dual AAVs. *Nat. Biotechnol.* 42, 253–264
100. Giehrl-Schwab, J. *et al.* (2022) Parkinson's disease motor symptoms rescue by CRISPRa-reprogramming astrocytes into GABAergic neurons. *EMBO Mol. Med.* 14, e14797
101. Shubina-Oleinik, O. *et al.* (2021) Dual-vector gene therapy restores cochlear amplification and auditory sensitivity in a mouse model of DFNB16 hearing loss. *Sci. Adv.* 7, eabi7629
102. Mich, J.K. *et al.* (2025) Interneuron-specific dual-AAV SCN1A gene replacement corrects epileptic phenotypes in mouse models of Dravet syndrome. *Sci. Transl. Med.* 17, eadn5603
103. Lim, C.K.W. *et al.* (2020) Treatment of a mouse model of ALS by in vivo base editing. *Mol. Ther. J. Am. Soc. Gene Ther.* 28, 1177–1189
104. Reever, C.M. *et al.* (2025) Base editing rescue of seizures and SUDEP in SCN8A developmental epileptic encephalopathy. *bioRxiv* Published online April 19, 2025. https://doi.org/10.1101/2025.04.09.647983
105. Conecker, G. *et al.* (2024) Global modified Delphi consensus on diagnosis, phenotypes, and treatment of SCN8A-related epilepsy and/or neurodevelopmental disorders. *Epilepsia* 65, 2322–2338
106. Dopeso-Reyes, I.G. *et al.* (2021) Editorial: tropism, mapping, modeling, or therapy using canine adenovirus type 2 (CAV-2) vectors in the CNS. *Front. Mol. Neurosci.* 14, 636476
107. Fadila, S. *et al.* (2023) Viral vector-mediated expression of $Na_V1.1$, after seizure onset, reduces epilepsy in mice with Dravet syndrome. *J. Clin. Invest.* 133, e159316
108. Frangoul, H. *et al.* (2021) CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. *N. Engl. J. Med.* 384, 252–260
109. Yu, W. *et al.* (2024) Allele-specific editing of a dominant SCN8A epilepsy variant protects against seizures and lethality in a murine model. *Ann. Neurol.* 96, 958–969
110. Gaudelli, N.M. *et al.* (2017) Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature* 551, 464–471
111. Anzalone, A.V. *et al.* (2019) Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature* 576, 149–157
112. Chen, P.J. *et al.* (2021) Enhanced prime editing systems by manipulating cellular determinants of editing outcomes. *Cell* 184, 5635–5652
113. Wettschurack, K. *et al.* (2024) Near 100% genome editing efficiency with a one-step all RNA prime editing system in a human reporter iPSC line. *bioRxiv* Published online December 21, 2024. https://doi.org/10.1101/2024.12.20.629762
114. Guo, R. *et al.* (2024) Engineered IscB-ωRNA system with improved base editing efficiency for disease correction via single AAV delivery in mice. *Cell Rep.* 43, 114973
115. Sousa, A.A. *et al.* (2025) In vivo prime editing rescues alternating hemiplegia of childhood in mice. *Cell* 188, 4275–4294
116. An, M. *et al.* (2024) Engineered virus-like particles for transient delivery of prime editor ribonucleoprotein complexes in vivo. *Nat. Biotechnol.* 42, 1526–1537
117. Banskota, S. *et al.* (2022) Engineered virus-like particles for efficient in vivo delivery of therapeutic proteins. *Cell* 185, 250–265
118. Liang, X. *et al.* (2025) Engineering of extracellular vesicles for efficient intracellular delivery of multimodal therapeutics including genome editors. *Nat. Commun.* 16, 4028
119. Geilenkeuser, J. *et al.* (2025) Engineered nucleocytosolic vehicles for loading of programmable editors. *Cell* 188, 2637–2655
120. Herrera-Barrera, M. *et al.* (2023) Lipid nanoparticle-enabled intracellular delivery of prime editors. *AAPS J.* 25, 65
121. Huang, H. *et al.* (2012) RNA editing of the IQ domain in Cav1.3 channels modulates their Ca^{2+}-dependent Inactivation. *Neuron* 73, 304–316
122. Ferrick-Kiddie, E.A. *et al.* (2017) Mutations underlying episodic ataxia type-1 antagonize Kv1.1 RNA editing. *Sci. Rep.* 7, 41095
123. Cox, D.B.T. *et al.* (2017) RNA editing with CRISPR-Cas13. *Science* 358, 1019–1027
124. Abudayyeh, O.O. *et al.* (2019) A cytosine deaminase for programmable single-base RNA editing. *Science* 365, 382–386
125. Lauffer, M.C. *et al.* (2024) Possibilities and limitations of antisense oligonucleotide therapies for the treatment of monogenic disorders. *Commun. Med.* 4, 6
126. Conroy, F. *et al.* (2022) Chemical engineering of therapeutic siRNAs for allele-specific gene silencing in Huntington's disease models. *Nat. Commun.* 13, 5802
127. Wagner, M. *et al.* (2025) Antisense oligonucleotide treatment in a preterm infant with early-onset SCN2A developmental and epileptic encephalopathy. *Nat. Med.* 31, 2174–2178
128. Neil, C.R. *et al.* (2025) Poison exons: tuning RNA splicing for targeted gene regulation. *Trends Pharmacol. Sci.* 46, 264–278
129. Seo, J.H. *et al.* (2023) DNA double-strand break-free CRISPR interference delays Huntington's disease progression in mice. *Commun. Biol.* 6, 466
130. Wang, J. *et al.* (2022) AAV-delivered suppressor tRNA overcomes a nonsense mutation in mice. *Nature* 604, 343–348
131. Dhindsa, R.S. *et al.* (2021) A transcriptome-based drug discovery paradigm for neurodevelopmental disorders. *Ann. Neurol.* 89, 199–211
132. Schust, L.F. *et al.* (2024) A patient organization perspective: charting the course to a cure for SCN2A-related disorders. *Ther. Adv. Rare Dis.* 5, 26330040241292645
133. Conecker, G. *et al.* (2025) Patient leadership and partnerships accelerate therapies for SCN8A and other developmental and epileptic encephalopathies. *Ther. Adv. Rare Dis.* 6, 26330040241252449
134. Kim-McManus, O. *et al.* (2024) A framework for N-of-1 trials of individualized gene-targeted therapies for genetic diseases. *Nat. Commun.* 15, 9802
135. Pan, X. *et al.* (2019) Molecular basis for pore blockade of human Na^+ channel Nav1.2 by the μ-conotoxin KIIIA. *Science* 363, 1309–1313