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Channelopathies in Epilepsy: from Precision Medicine to Gene Therapy

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Abstract. Epilepsy is a common and debilitating neurological disease with a significant genetic component. Inherited or de novo mutations in ion channels resulting in periodic network hyperexcitability is a major cause of epileptogenesis. Understanding of the genetic etiology of epilepsy opens up the avenue of personalized treatment. A range of precision medications are designed to target the defective ion channels, with varied effects. In addition, the underlying mechanisms of channelopathies can also be addressed by gene therapy. Expression of mutated ion channel genes can be regulated via gene replacement or gene silencing approaches, including viral vector-mediated gene replacement and antisense oligonucleotides. Aside from directly compensating of ion channel dysfunction, another strategy of gene therapy involves the overexpression of certain ion channels to modulate neuronal excitability, which particularly targets focal epilepsies. In line with the paroxysmal nature of epileptic seizures, optogenetic and chemogenetic approaches, which permits conditional activation of gene products can be particularly beneficial. The ideal gene therapy to treat genetic epilepsies and channelopathies would be in vivo gene editing, making precise gene modifications to correct mutations harbored by each individual. Advances in CRISPR-Cas technology has brought the idea closer to reality, yet technical and ethical concerns surround its therapeutic application.

Keywords: epilepsy, channelopathy, gene therapy, gene editing

1. Introduction

Epilepsy is a chronic condition characterised by recurrent seizures. It is not only one of the world's oldest recognised diseases, but also a present-day health imperative, affecting over 50 million people of all ages and sexes worldwide [1]. Based on international epidemiological studies, every 7.6 per 1000 individuals will experience epilepsy at certain point in life, with the highest incidence occurring in younger and older age groups [2]. The World Health Organization's 2016 study ranks epilepsy as the third most burdensome neurological disease [3]. Affected individuals usually experience not only the physical symptoms of seizure attacks, but various other psychiatric comorbidities as well, such as depression and anxiety, which further reduce the quality of life. Moreover, epilepsy patients carry three-fold higher risk of premature death, which can occur either as a direct consequence of prolonged uncontrolled seizures, or due to indirect causes such as drowning [1].

Epileptic seizures are characterized by abnormal neuronal hyperactivity in the brain. They can be broadly divided into two types: focal (originating from a local neuronal network in one hemisphere) or generalized (originating in both hemispheres simultaneously) seizures. The causes of epilepsy

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encompass various pathogenic events that perturb normal brain function, including structural, infectious, immune, metabolic and genetic aetiology [4]. It is believed that genetic factors are implicated in 70-80% of epilepsies [5].

Since the first epileptic gene was identified in 1995, research in epilepsy genetics progressed remarkably, having transformed from early stage of gene discovery in familial epilepsies to an era of rapid gene discovery via next-generation sequencing. To date, about 1000 genes have been related to epilepsy [6]. They exhibit varied inheritance patterns and degrees of genotype-phenotype correlations and implicate a broad range of pathological mechanisms. Genetic channelopathies constitute a large proportion of epilepsy despite the increasingly complex genetic architecture of the disease.

Antiseizure drugs (ASDs) are the current mainstay of treatment for epilepsy. However, a third of patients continue to have uncontrolled seizures despite having access to dozens of ASDs. Furthermore, the introduction of multiple new drugs with diverse mechanisms of action over the past two decades has not increased the proportion of seizure-free patients [7]. Doubtlessly, epilepsy is calling for novel therapeutic strategies, and growing understanding of the genetic aetiology of epilepsy could insight into the development of personalized therapeutic strategies such as precision medicine, addressing the underlying mechanism of epileptogenesis.

Gene therapy approaches have moved from theory to clinical practice and have been approved for several neurological diseases [8]. Current viral vector-based gene therapy permits cell-specific modification of neuronal excitability, making it a promising candidate for antiepileptic treatment. Furthermore, rapid advances in CRISPR technology could usher in a new era of treatment that repairs disease-causing mutations directly at genomic level.

This essay will briefly review mutations in different types of ion channels that have been implicated in epilepsy and discuss how they could be targeted by precision medicine before discussing gene therapy as another option of personalized therapy.

2. Genetic channelopathies in epilepsy

Genetic channelopathies refer to the diseases caused by genetic dysfunction of ion channels. Ion channels are transmembrane proteins facilitating the transport of ions down their concentration gradient across the lipid bilayer. They typically exhibit selectivity towards the passage of specific ions, and can be gated, allowing the passage of ions to be controlled by change in electric potential (voltage-gated), the binding of a particular ligand (ligand-gated) or other factors. Widely expressed in the CNS, ion channels have crucial roles in the regulation of membrane potential, the generation and propagation of action potentials and synaptic transmission. Thus, ion channel dysfunctions typically cause impaired membrane excitability, which can lead to the generation of epileptic seizures. Indeed, the earliest identified epilepsy-causing genes were those encoding ion channels, and epilepsy was once believed to be a family of genetic channelopathies. The advent of new genomic technologies such as next generation sequencing has led to increasing number of epilepsy-causing genes being discovered rapidly, allowing our knowledge of the genetic architecture of epilepsy to expand beyond the ion channels [5]. Nevertheless, ion channel mutations are still the major contributor to a range of epilepsy syndromes, estimated to be responsible for about 25% of monogenic epilepsies [9]. Ion channels are critical for regulating neuronal excitability and modulating synaptic transmission. Thus, they are implicated in critical pathways for epileptogenesis and are promising targets for both precision medicine and potential gene therapies. Of note, epileptic encephalopathies refer to a variety of syndromes where epileptic activity leads to the severe cognitive and behavioural impairment, beyond what is expected from the underlying pathology alone [4]. Channelopathies are typically associated with early-onset epileptic encephalopathies, which tend to be life-threatening and are often accompanied by developmental disorders such as autism spectrum disorders and intellectual disability. The next part of this article will outline the roles of some of the most studied ion channels in epileptogenesis and the associated epilepsy syndromes, followed by precision medications that target them.

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2.1. Ion channels modulating neuronal excitability

2.1.1. Voltage-gated sodium channels. Voltage-gated sodium channels are vital for the generation and propagation of axon potentials by allowing for rapid influx of Na+ upon membrane depolarization. Pathogenic mutations associated with epilepsy have mostly been identified in genes encoding the pore-forming α-subunits: SCN1A, SCN2A, SCN3A and SCN8A.

SCN1A is one of the most established causative gene in epilepsy. It encodes the Nav1.1 subunit. Pathogenic mutations in SCN1A are predominantly loss-of-function (LoF) and causes haploinsufficiency. The result is a reduced Na+ current. As Nav1.1 is mainly expressed in GABAergic inhibitory neurons, this leads to reduced inhibition and thus a hyperexcitable neuronal network. As a typical example of monogenic epilepsy, SCNA1 mutations account for over 80% of cases of Dravet syndrome, a severe early-onset epileptic encephalopathy [10]. In contrast, epilepsy-associated mutations SCN2/3/8A are predominantly gain-of-function (GoF) variants. It is suggested that these variants have a greater impact on excitatory neurons than on inhibitory ones due to their higher expression level in the former. These mutations usually lead to early-infantile epileptic encephalopathies, with a high incidence of sudden death [10].

2.1.2. Voltage-gated potassium channels. As the most diverse group of ion channels expressed in the central nervous systems, there are different types of potassium channels involved in the fine tuning of action potentials.

Voltage-gated potassium channels are the largest group and the main focus in epilepsy research amongst all potassium channels. They consist of 12 subfamilies of pore-forming α-subunits encoded by about 80 genes, over 10 of which have been implicated in epilepsy [11]. The pathogenic variants show varied degrees of connection to a spectrum of epileptic encephalopathies, and the underlying cellular mechanisms are often not fully understood [9]. KCNQ2/3 mutations have been the most studied and their correlations to epilepsy syndromes are believed to be the strongest [12]. KCNQ2/3 encode two subunits of the Kv7 subfamily: Kv7.2 and Kv7.3 respectively. They assemble to form the slow activating voltage gated potassium channel that regulate the slow non-inactivating M current. The voltage-depending M current is essential to constrain the high-frequency firing of action potential, thus preventing the abnormal bursting activity of neurons [11]. LoF mutations in the two genes give rise to a wide range of epilepsy syndromes, from milder self-limited (formerly referred to as benign) familial neonatal epilepsy (BFNE) to more severe early-onset epileptic encephalopathies [13]. It is self-evident that decreased M-current caused by loss of Kv7.2/3 function in excitatory neurons should lead to prolonged depolarization. However, it is found that KCNQ2/3 deletion in inhibitory neurons also results in network hyperexcitability through the mechanism of homeostatic potentiation of excitatory transmission [14].

2.1.3. Sodium-activated potassium channels. Specific epilepsy syndromes are also associated with mutations in KCNT1, which encodes KNa1.1. The affected ion channels are thought to be involved in modulating hyperpolarization after repetitive firing of action potentials. GoF variants of KCNT1 have been identified as the genetic cause of a range of epilepsy syndromes, including epilepsy of infancy with migrating focal seizures (EIMFS) and autosomal-dominant nocturnal frontal lobe epilepsy (ADNFLE) [15].

2.2. Ion channels modulating synaptic transmission

2.2.1. GABAA receptors. GABA is the major inhibitory neurotransmitter in the CNS and GABAA receptors are widely expressed. Upon ligand binding GABAA receptors lead to a brief influx of chloride ions at the postsynaptic membrane, thereby mediating the phasic inhibition. LoF mutations in GABRA1, GABRG2 and GABRB3, which encode α, γ and β subunits of the GABAAR respectively, have been associated with a range of epilepsy syndromes, from genetic generalized epilepsy to more severe

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epileptic encephalopathies (e.g., Dravet syndrome). These mutations not only cause dysfunction of GABAAR, but impair the trafficking of receptor as well, together leading to abnormal GABAergic neurotransmission and generation of seizures [16,17].

2.2.2. N-Methyl-D-Aspartate (NMDA) glutamate receptors. NMDA receptors are non-selective cation channels mediating excitatory neurotransmission. They are permeable to calcium as well as Na+ and potassium ions. NMDARs are composed of both glycine-binding (GluN1) and glutamate-binding (GluN2) subunits. Pathological mutations in epilepsy have most frequently been identified in GRIN1A, which encodes GluN2A subunit. GRIN2A variants exhibit a wide range of epileptic phenotypes, including early-onset epileptic encephalopathies and atypical rolandic epilepsy (i.e., childhood epilepsy with centrotemporal spikes) [19].

In general, great phenotypic heterogeneity can be observed for every epilepsy-associated ion channel gene and genetic heterogeneity occurs for every epilepsy syndrome. As discussed above, all the genes implicated in channelopathies exhibit some degree of phenotypic pleiotropy. Different variants may have different clinical manifestations, from severe early-onset epileptic encephalopathies to milder adult-onset epilepsies, involving both generalized and focal seizures. Phenotypes may also differ with age. Epilepsy with early onset in infancy or childhood may be modified into different syndromes in adulthood. Furthermore, heterogeneous genetic aetiology is shown to underly the same phenotypes. Even for Dravet syndrome, where 80% of cases arise due to monogenic mutation in SCN1A, GABAA receptor genes still play causative roles in some cases. Studies on epileptic genetics have focused on not only inherited variants in familial cases but also the identification of de novo variants, which have been associated with most channelopathies discussed above. However, although the correlations between these ion channel genes and rare specific epilepsy syndromes (e.g., Dravet syndrome) have been established, there is less knowledge of the genetic landscape of more common epilepsies. It is believed that these epilepsies are caused by a contribution of multiple susceptible genes with different inheritance patterns, or de novo mutations [6]. Nevertheless, the identification of the causative ion channel mutations in epilepsy have provided new insights on treatment targets.

2.3. Precision medications

Pharmacological therapy is the current mainstay of treatment for epilepsy. Despite the availability of a wide range of ASDs with different mechanisms of action, a third of patients continue to have uncontrolled seizures. With increasing understanding of the causative epilepsy genes, the administration of personalised medications, which aims to target the treatment to each patient's specific aetiology, has been envisioned. In terms of channelopathies, there have been advances in repurposing existing drugs to target the mutated ion channels.

2.3.1. Sodium channel blockers. A number of existing ASDs act by blocking voltage-gated Na+ channels, such as carbamazepine, phenytoin and lamotrigine [10]. Na+ channel blockers (SCBs) have dichotomous roles in addressing epilepsy syndromes caused by Na+ channel mutations.

On the one hand, for Dravet syndrome and other SCN1A-related epilepsies, SCBs typically exacerbate seizures and worsen the symptoms because the syndromes themselves result from loss of Na+ channel function. Although some ASDs, such as cannabidiol have shown selective efficacy against SCN1A-related epilepsy, the mechanism of action does not address the underlying genetic defect [20].

On the other hand, SCN2A- and SCN8A-related epilepsies show responsiveness to SCBs. For example, phenytoin has been found to exhibit high efficacy towards SCN8A-related drug-resistant epilepsy in a recent case report [21]. Hence, SCBs could be a potential choice of personalized medicine targeting GoF mutations in SCN2A and SCN8A.

2.3.2. Potassium channel blockers and openers. Quinidine has been reported to counteract the GoF mutations in KCNT1-related epilepsies via the mechanism of blocking the mutated Na+-activated potassium channels [15]. However, studies aiming to demonstrate the clinical efficacy of quinidine as a

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precision medicine have produced mixed results [15,22]. It remains contradictory whether quinidine could produce better treatment outcome compared to non-personalized ASDs. The possible reasons could that the different patients in case reports harbour different KCNT1 mutations and thus respond to the same drug differently.

Ezogabine could act as a selective opener for voltage-gated potassium channels mediating the M-current and thus was considered as personalized medicine for patients with loss of KCNQ2 function [23]. However, the drug has been removed from market due to adverse side effects observed in patients. More recently, gabapentin has been found to be a potent activator of M-current, making it a possible drug candidate for KCNQ2/3-related epilepsy [24].

2.3.3. NMDA receptor antagonists. NMDA receptor antagonists such as memantine can reduce the overstimulation of NMDA receptors, and thus have been suggested as potential precision medicine for GRIN2A- and GRIN2B-related epilepsies [25].

Indeed, the above channel blockers and openers, whose efficacy have been shown in various case reports, have demonstrated the potential of personalized medicine for epilepsy caused by channelopathies. However, the responses to these drugs have proved variable and, in some cases, unsatisfactory. The complex clinical response pattern can be due to various confounding factors, including the design of clinical trials and the criteria to assess drug efficacy. Moreover, some of the drugs have been associated with serious side effects, suggesting potential safety concerns regarding the use of ASDs.

In addition to repurposing existing drugs to target the mutant ion channel proteins, another avenue of personalised therapy for these channelopathies involves direct targeting at the affected DNA or RNA, which can be achieved by gene therapy approaches.

3. Gene therapy

Gene therapy has become a critical treatment option for a variety of inherited or acquired genetic disorders. Recent approval of a gene replacement therapy, Zolgensma, as a single-dose treatment for spinal muscular atrophy marks a major milestone in the application of gene therapy in neurological disorders [26]. Currently, several different approaches are being used, including viral-vector mediated delivery of exogenous gene and RNA interference.

In classical gene replacement therapy, the efficacy and safety of transgene expression largely depend on the use of proper viral vectors which effectively transduce host cells without inducing significant immune response. At present, the two main classes of vectors being used are adeno-associated virus (AAV) and lentivirus, with respective advantages and disadvantages. Lentiviral vectors can integrate into the human genome, which allows persistent transgene expression while posing the risk of insertional mutagenesis. In contrast, exogenous DNA delivered by AAV vectors can exist as episome and does not integrate into the genome under most circumstances, reducing the risk of mutation associated with viral integration. However, the former has much larger packing capacity and allows for more spatially restricted transgene expression. In addition to transgene delivery, the success of gene therapy is also dependent upon the use of specific promotors to limit transgene expression to specific types or subtypes of neurons [27]. In addition, the expression of a target gene can also be modulated by RNA interference. This is typically achieved by an antisense oligonucleotide (ASO), whose sequence is complementary to the targeted mRNA. This permits specific modulation of gene expression with high specificity by targeting mRNA processing [8]. This non-viral mediated gene therapy using ASOs bypasses the disadvantages of viral vectors. However, the short, single-stranded RNA have limited half-life and thus usually require regular administration.

Gene therapy for monogenic diseases typically aims to compensate the disease mechanism (LoF or GoF) by down or upregulating gene expression. In terms of antiepileptic treatment, although gene therapy has not entered everyday clinical practice, the same principles apply. Proof-of-concept studies using animal models have demonstrated the effectiveness of multiple approaches. These include not only those compensating ion channel mutations directly, but also those targeting neuronal excitability

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via overexpression of ion channels, which has mainly been developed for focal epilepsies. Furthermore, optogenetic and chemogenetic approaches confer flexibility to the activation of gene therapy products, permitting on-demand regulation of network excitability. Finally, the development of gene-editing technology holds great promise to be employed in the future to perform highly targeted gene therapies that directly modify mutated genes.

3.1. Targeting mutated ion channels

There is growing interest in developing gene therapies targeting specific genetic aetiology of epilepsy, such as ion channel mutations. Research in this field has just started and has yielded relatively little outcome. Significant hurdles to research progress include the rarity of epileptic encephalopathies, the lack of established phenotypic specificity. One exception is Dravet syndrome, which is almost exclusively caused by SCN1A LoF. Indeed, as can be seen from the examples below, SCN1A is the most-actively studied target of antiepileptic gene therapy amongst all the ion channel genes associated with epilepsy. The large sizes of ion channel genes also render gene replacement therapy impractical for most channelopathies as the packing capacity of viral vectors are limited. Nevertheless, there are still a range of proof-of-concept antiepileptic gene therapy targeting channelopathies.

3.1.1. Targeting SCN1A. As previously described, the life-threatening Dravet syndrome is predominantly caused by LoF SCN1A mutation, resulting in Nav1.1 haploinsufficiency that mainly affects inhibitory interneurons. The largely monogenic nature renders it a perfect candidate for gene therapy.

RNA interference approaches aim to upregulate the expression of endogenous wild-type SCN1A allele. One strategy that has entered clinical trial uses ASOs to modulate the splicing of pre-mRNA. It prevents the naturally occurring non-productive alternative splicing events, thus increasing the production of functional mRNA [28]. Another ASO-mediated approach targets an endogenous antisense non-coding RNA that naturally represses the expression of SCN1A. The ASOs are therefore able upregulate expression of the healthy SCN1A allele by inhibiting the repressive RNA [29]. Both approaches successfully reduce seizure frequency in mice models. However, the durability of these ASOs have shown to be limited. Hence injection should be performed on a regular basis to achieve constant effect.

Delivery of an exogenous functional copy of SCN1A is not feasible because the size of SCN1A gene (over 6kb) exceeds the packing capacity of AAVs (4.7kb). Although lentivirus has large enough capacity, it is not suitable for mutations affecting large brain areas due to its limited spread. One innovative strategy was developed to circumvent the obstacle of traditional gene replacement therapy by delivering a transcription factor, which is small enough to be packed into an AAV vector. It is targeted to inhibitory interneurons, where it upregulates the expression of SCN1A [30].

3.1.2. Targeting SCN2/8A. A recent study has demonstrated the effectiveness of an ASO-based approach in delaying seizure onset and death in a mouse model harbouring patient-based SCN8A GoF mutation [31]. Similarly, an ASO designed to downregulate SCN2A expression in SCN2A GoF mice was also shown to have translational potential in reducing seizures [32].

3.1.3. Targeting GABRG2. GABRG2 LoF, which accounts for a small proportion of Dravet syndrome, has also been investigated as a potential target of RNA interference. An aminoglycoside, gentamicin, was found to partially restore the synthesis of full-length GABAAR γ2 subunit by inducing mRNA readthrough, bypassing the premature stop codon caused by a specific LoF variant [33].

3.2. Overexpression of ion channels

Instead of targeting specific pathogenic mutations, a vast body of research on anti-epileptic gene therapy has targeted specific epileptogenic zones. Indeed, viral vectors usually require stereotactic injection into the CNS to bypass the blood-brain barrier. Therefore, their spatially restricted transduction renders them

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a natural candidate for focal epilepsies with discreet regions of seizure onset. Gene therapy approaches for these focal epilepsies rely on modulating neuronal excitability within the seizure foci, which is mainly achieved by the overexpression of specific ion channels. Of note, these approaches do not specifically target the etiology of epilepsy. Instead, they are designed to target seizure foci. Nevertheless, it is possible that these focally delivered gene therapies could be modified to benefit other types of epilepsy should they prove safe and efficacious in the future. In theory, for example, they can also be applied to modulating network or circuit excitability in channelopathies.

3.2.1. GABRA1 overexpression. Temporal lobe epilepsy is the most common adult-onset acquired epilepsy [34]. AAV-mediated delivery of exogenous GABRA1 to a specific region of temporal lobe has been reported to significantly decrease the number of recurrent seizures by 60%, preventing the development of temporal epilepsy [34]. This is mainly due to the impact of GABAAR subunit composition on the inhibitory signaling mediated by the receptor [35]. The overexpression of GABAAR α1 subunit in the seizure focus has the effect of restoring inhibitory signaling mediated by chloride ion influx, thus rebalancing excitation and inhibition to prevent epileptogenesis.

3.2.2. KCNA1 overexpression. Alternatively, overexpression of potassium channels genes can also be decrease the neuronal hyperexcitability. Recently, Snowball et al. [36] have demonstrated that viral vector mediated delivery of KCNA1 gene to seizure foci also suppresses seizure generation in neocortical epilepsy. The use of a cell-type specific promoter to drive transgene expression only in excitatory neurons as well as a lentiviral vector to minimized mutagenesis marks a great advance in the development of an effective cell-type specific, locally restricted gene therapy. Of note, the same construct was shown to be effective against temporal lobe epilepsy as well when packaged into an AAV vector injected to the respective seizure focus [36].

3.3. Optogenetics and chemogenetics

Viral vector-based gene therapies share several limitations. These include the viral-induced immune response and insertional mutagenesis, which are being addressed by the development of more effective viral vectors. More selective promoters are also being developed to increase the currently limited specificity of transgene expression, as mentioned above. In addition, there are further safety concerns. One major problem regards its permanency [8]. Currently, the number of gene copies transported into a neuron cannot be controlled, thus the long-term overexpression of the gene product itself may exert adverse effect on the cell. Aside from using RNA interference-based approaches, another way to reduce such risk is to utilize optogenetic or chemogenetic approaches to control the activation of gene product. In this way, the neuronal network can operate normally between the episodic onset of seizure attacks.

Optogenetics relies on the expression of opsin genes derived from microorganisms. These genes encode light-activated inhibitory or excitatory ion channels, which can be targeted by viral vector constructs to selective neurons to regulate networks excitability in replacement of human DNA [37]. Although it permits rapid activation of the exogenous ion channel depending on the light stimulation, implanting light source into the brain is also invasive. In addition, the expression of microbe-derived protein further imposes the risk of inducing immune response.

In contrast, chemogenetic approach utilises designer receptors exclusively activated by designer drugs (DREADDs) that are human proteins in nature [38]. These proteins are targeted to specific cell types by viral vectors and are engineered to be activated only by exogenous ligands rather than endogenous ligands. Although they provide control over transgene expression and delivery of the ligand typically by non-invasive oral administration, chemogenetic approach has a major disadvantage, which is the slowness in receptor activation, which can take hours [38].

3.4. Gene editing

Recent advances in the development of CRISPR-Cas technology have greatly changed the landscape of genome-editing. The technology confers unprecedented simplicity and cost-effectiveness to the

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conduction of site-specific genome editing. Derived from the adaptive immune system naturally evolved in prokaryotes, CRISPR-Cas system used for gene-editing is a protein-RNA complex composed of a guide RNA sequence that targets the complex to the desired DNA sequence and a Cas protein, such as Cas9 that cleaves the double strand. The repair of the double-strand break is carried out by either non-homologous end joining (NHEJ) or homology-directed repair (HDR). The former frequently introduces errors while the latter achieves accurate repair with the presence of a DNA template [39]. Gene editing can ideally overcome the major disadvantages of the forementioned gene therapy approaches, by precisely modifying pathogenic gene mutations. It holds great promise as an individualized antiepileptic gene therapy tool, especially for the rare and severe drug-resistant channelopathies.

However, therapeutic gene editing still faces great challenges. The delivery of CRISPR-Cas toolbox presents an initial barrier against successful in vivo CRISPR. To perform HDR, a DNA template containing the homologous sequence immediately upstream and downstream the mutation site needs to be delivered together with the CRISPR-Cas system precisely into the target cell, which imposes a challenge on the development of proper vectors [27]. Another technical problem associated with genome editing is the lack of control over off-target mutations, which is caused by the off-target activity of designed guide RNA [39]. For highly structured proteins such as ion channels, it is crucial to ensure the protein function is not affected. Thus, clinical application of in vivo CRISPR would only be rendered safe if these off-target mutations could be avoided or at least predicted. Finally, a large proportion of epilepsy encephalopathies and severe epilepsy syndromes associated with channelopathies are early-onset and thus requires gene editing early in development or even germline gene editing, raising potential ethical concerns which in part stem from the unresolved technical and safety problems.

Indeed, the development of gene editing therapy is still in its nascent stage. Nevertheless, the application of CRISPR-Cas system is not limited to direct base editing. Recently, Colasante et al. [40] have used mouse model to show that AAV-mediated delivery of CRISPR activation tool could effectively upregulate the expression of endogenous KCNA1 in excitatory neurons. This variant of CRISPR system uses defective Cas9 protein with inactivated nuclease activity to bind a transcription activator. Cas9 is targeted to the promoter of the DNA sequence, where transcription activator acts to upregulate gene expression. CRISPR mediated regulation of gene expression further opens a new avenue of gene therapy.

4. Conclusion

The past few decades have witnessed substantial progress in epilepsy research and great advances in our understanding of various aspects of the disease. With more and more emphasis being placed on the etiology of epilepsy, genetic background of the disease has been gradually unveiled, with important findings not only boosting the development of basic science, but also being integrated into developing innovative treatment options. Currently, treatment outcome of ASDs is limited, especially for epileptic encephalopathies caused by mutations in ion channels. To improve the effectiveness of drug treatment, a series of channel openers and blockers has been developed as precision medicine for these channelopathies. Gene therapy opens up another avenue of personalized treatment of epilepsy. Approaches that directly target mutated ion channels have been investigated predominantly in monogenic Dravet syndrome. Other channelopathies and associated epileptic syndromes exhibit much less genotypic and phenotypic specificity, and the underlying pathogenic mechanisms are less understood. Thus, the development of gene-specific therapy that directly compensate these mechanisms has lagged. An alternative strategy of gene therapy directly mediates neuronal excitability in epileptogenic foci. Optogenetic and chemogenetic approaches further provide effective 'switch' controlling the activation transgene expression products. In the future, the most ideal gene therapy would possibly be carried out by CRISPR-mediated gene editing, allowing different mutations of each individual to be specifically targeted by reprogrammable guide RNA. Future research is needed to further establish the genetic architecture of epilepsy while continuing to explore the potential and approaches of gene therapy in treating the diseases. Alhough antiepileptic gene therapy remains in preclinical stage at present, it holds great promise to usher in a new era of epilepsy treatment, not only

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for channelopathies, but for epilepsies with unclear genetic causes as well, which can be achieved by overexpression of ion channels to modulate network excitability.

References

[1] World Health Organization. Epilepsy: a public health imperative. World Health Organization, 2019.

[2] K. M. Fiest, et al., "Prevalence and incidence of epilepsy: a systematic review and meta-analysis of international studies." *Neurology* **88,** 3, 296-303 (2017).

[3] V. L. Feigin, et al., "Global, regional, and national burden of neurological disorders, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016." *The Lancet Neurology*,**18**, 5, 459-480 (2019).

[4] E. Scheffer, et al., "ILAE classification of the epilepsies: position paper of the ILAE commission for classification and terminology." *Epilepsia*, **58**, 512-521 (2017).

[5] C.T. Myers, & H.C. Mefford, "Advancing epilepsy genetics in the genomic era." *Genome Medicine*, **7,** 1 (2015).

[6] K. M. J. Van Loo, et al., "Epigenetic genes and epilepsy — emerging mechanisms and clinical applications." *Nature Reviews Neurology* (2022).

[7] Z. Chen, et al., "Treatment outcomes in patients with newly diagnosed epilepsy treated with established and new antiepileptic drugs: a 30-year longitudinal cohort study." *JAMA neurology* **75**, 3, 279–286 (2017).

[8] D. Brenner, A. C. Ludolph & J. H. Weishaupt, "Gene specific therapies – the next therapeutic milestone in neurology." *Neurological Research and Practice*, **2**, 25 (2020).

[9] J. Oyrer, et al., "Ion channels in genetic epilepsy: From genes and mechanisms to disease-targeted therapies." *Pharmacological Reviews*, **70**, 1, 142–173 (2018).

[10] Brunklaus & D. Lal, "Sodium channel epilepsies and neurodevelopmental disorders: from disease mechanisms to clinical application." *Developmental Medicine and Child Neurology*, **62**, 7, 784–792 (2020).

[11] N. M. Allen, et al. "Genetic potassium channel-associated epilepsies: clinical review of the Kv family." *European Journal of Paediatric Neurology*. **24**, 105-116 (2020).

[12] E. S. Nikitin & L.V. Vinogradova, "Potassium channels as prominent targets and tools for the treatment of epilepsy." *Expert Opinion on Therapeutic Targets*, **25**, 3, 223–235 (2021).

[13] M. Kuersten, et al., "Antiepileptic therapy approaches in KCNQ2 related epilepsy: a systematic review." *European Journal of Medical Genetics*, **63**, 1 (2020).

[14] H. Soh, et al., "Deletion of KCNQ2/3 potassium channels from PV+ interneurons leads to homeostatic potentiation of excitatory transmission." **eLife**, **7**, e38617 (2018).

[15] F. Borlot, et al., "KCNT1-related epilepsy: an international multicenter cohort of 27 pediatric cases." *Epilepsia*, **61**, 4, 679–692 (2020).

[16] Ghit, D. Assal, A. S. Al-Shami & D. E. E. Hussein, "GABAA receptors: structure, function, pharmacology, and related disorders." *Journal of Genetic Engineering and Biotechnology*, **19**, 1 (2021).

[17] M. Mele, R. O. Costa & C. B. Duarte, "Alterations in GABAA-receptor trafficking and synaptic dysfunction in brain disorders." *Frontiers in Cellular Neuroscience*, **13** (2019).

[18] W. XiangWei, Y. Jiang & H. Yuan, "De novo mutations and rare variants occurring in NMDA receptors." *Current Opinion in Physiology*, **2**, 27-35 (2018).

[19] Devinsky, et al., "Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome." *New England Journal of Medicine*, **376**, 21 (2017).

[20] R. S. Boerma, et al., "Remarkable phenytoin sensitivity in 4 children with SCN8A-related epilepsy: a molecular neuropharmacological approach." *Neurotherapeutics*, **13**, 1, 192-197 (2016).

[21] M. P. Fitzgerald, et al., "Treatment responsiveness in KCNT1-related epilepsy." *Neurotherapeutics*, **16**, 3, 848-857 (2019).

DOI: 10.54254/2753-8818/3/20220298

[22] J. J. Millichap, et al., "KCNQ2 encephalopathy: features, mutational hot spots, and ezogabine treatment of 11 patients." *Neurology: Genetics,* **2**, 5 (2016).
[23] R. W. Manville & G. W. Abbott, "Gabapentin is a potent activator of KCNQ3 and KCNQ5 potassium channels." *Molecular Pharmacology*, **94**, 4, 1155–1163 (2018).
[24] T. M. Pierson, et al., "GRIN2A mutation and early-onset epileptic encephalopathy: personalized therapy with memantine." *Annals of Clinical and Translational Neurology*, **1**, 3, 190-198 (2014).
[25] National Institute for Health and Care Research., "AVXS-101 for spinal muscular atrophy." NIHR innovation observatory evidence briefing, 1-9 (2018).
[26] C. E. Dunbar, K. A. High, J. K. Joung, D. B. Kohn, K. Ozawa, & M. Sadelain, "Gene therapy comes of age." *Science*, **359**, eaan4672 (2018).
[27] Z. Han, et al., "Antisense oligonucleotides increase Scn1a expression and reduce seizures and SUDEP incidence in a mouse model of Dravet syndrome." *Science Translational Medicine*, **12**, eaaz6100 (2020).
[28] J. Hsiao, et al., "Upregulation of haploinsufficient gene expression in the brain by targeting a long non-coding RNA improves seizure phenotype in a model of Dravet syndrome." *EBioMedicine*, **9**, 257-277 (2016).
[29] Tanenhaus, et al., "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." *Human Gene Therapy,* **33**, 11-12, 579-597 (2022).
[30] G. M. Lenk, et al., "Scn8a antisense oligonucleotide is protective in mouse models of SCN8A encephalopathy and Dravet syndrome." *Annals of Neurology*, **87**, 3, 339-346 (2020).
[31] M. Li, et al. "Antisense oligonucleotide therapy reduces seizures and extends life span in an SCN2A gain-of-function epilepsy model." *The Journal of Clinical Investigation*, **131**, 23, e152079 (2021).
[32] X. Huang, M. Tian, C. C. Hernandez, N. Hu & R. L. MacDonald, "The GABRG2 nonsense mutation, Q40X, associated with Dravet syndrome activated NMD and generated a truncated subunit that was partially rescued by aminoglycoside-induced stop codon read-through." *Neurobiology of Disease*, **48**, 1, 115–123 (2012).
[33] Y. S. H. Raol, I. V. Lund, S. Bandyopadhyay, G. Zhang, D. S. Roberts, J. H. Wolfe, S. J. Russek & A. R. Brooks-Kayal, "Enhancing GABAA receptor α1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal lobe epilepsy." *Journal of Neuroscience*, **26**, 44, 11342–11346 (2006).
[34] E. Tipton & S. J. Russek, "Regulation of inhibitory signaling at the receptor and cellular Level; Advances in our understanding of GABAergic neurotransmission and the mechanisms by which it is disrupted in epilepsy." *Frontiers in Synaptic Neuroscience*, **14** (2022).
[35] Snowball, et al., "Epilepsy gene therapy using an engineered potassium channel." *Journal of Neuroscience*, **39**,16, 3159–3169 (2019).
[36] R. C. Wykes, D. M. Kullmann, I. Pavlov & V. Magloire "Optogenetic approaches to treat epilepsy." *Journal of Neuroscience Methods*, **260**, 215–220 (2016).
[37] J. Magnus, et al., "Ultrapotent chemogenetics for research and potential clinical applications." **Science**, **364** (2019).
[38] S. Nidhi, U. Anand, P. Oleksak, P. Tripathi, J. A. Lal, G. Thomas, K. Kuca & V. Tripathi, "Novel crispr–cas systems: An updated review of the current achievements, applications, and future research perspectives." *International Journal of Molecular Sciences*, **22,** 7 (2021).
[39] G. Colasante, et al., "In vivo CRISPRa decreases seizures and rescues cognitive deficits in a rodent model of epilepsy." *Brain*, **143**, 3, 891–905 (2020).