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# Promoting CRISPRa for Targeted Treatment of Epilepsy

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**In Vivo CRISPRa Decreases Seizures and Rescues Cognitive Deficits in a Rodent Model of Epilepsy**

Colasante G, Qiu Y, Massimino L, et al. *Brain*. 2020;143(3):891-905. doi:10.1093/brain/awaa045

Epilepsy is a major health burden, calling for new mechanistic insights and therapies. CRISPR-mediated gene editing shows promise to cure genetic pathologies, although hitherto it has mostly been applied ex vivo. Its translational potential for treating nongenetic pathologies is still unexplored. Furthermore, neurological diseases represent an important challenge for the application of CRISPR because of the need in many cases to manipulate gene function of neurons in situ. A variant of CRISPR, CRISPRa, offers the possibility to modulate the expression of endogenous genes by directly targeting their promoters. We asked if this strategy can effectively treat acquired focal epilepsy, focusing on ion channels because their manipulation is known to be effective in changing network hyperactivity and hypersynchronization. We applied a doxycycline-inducible CRISPRa technology to increase the expression of the potassium channel gene *Kcna1* (encoding Kv1.1) in mouse hippocampal excitatory neurons. CRISPRa-mediated Kv1.1 upregulation led to a substantial decrease in neuronal excitability. Continuous video electroencephalogram telemetry showed that AAV9-mediated delivery of CRISPRa, upon doxycycline administration, decreased spontaneous generalized tonic–clonic seizures in a model of temporal lobe epilepsy and rescued cognitive impairment and transcriptomic alterations associated with chronic epilepsy. The focal treatment minimizes concerns about off-target effects in other organs and brain areas. This study provides the proof-of-principle for a translational CRISPR-based approach to treat neurological diseases characterized by abnormal circuit excitability.

**Keywords**
acquired epilepsy, CRISPR, focal epilepsy, potassium channel, TLE

## Commentary

The genome modifying mechanism CRISPR (clustered regularly interspaced short palindromic repeats) lets scientists edit specific sequences of DNA[1] and provides a new strategy for the development of precision treatments for a wide range of diseases.[2] The CRISPR-Cas system utilizes short guide RNAs (sgRNAs) to direct site-specific binding of the endonuclease Cas9. The 2 cleavage domains of Cas9 catalyze a double-stranded DNA break. Repair via nonhomologous end joining introduces insertions or deletions that inactivate a targeted gene. Alternatively, homology-directed repair can use a provided template to knock-in a desired sequence or to correct genetic mutations.

Manipulation of Cas9 nuclease activity has enabled broader uses of the CRISPR-Cas system. Inactivation of one cleavage domain creates a Cas9 nickase. The nickase produces single-stranded DNA breaks that are repaired with high fidelity by the base excision repair pathway. Paired nickase strategies can minimize off-target effects and improve the rate of homology-directed repair.[3] Inactivation of both cleavage domains yields a catalytically inactive "dead" Cas9 (dCas9) that retains the ability to bind to target DNA sequences but does not cut DNA.[4] Recently, dCas9 has been used in conjunction with transcriptional activators or repressors to modify the expression of endogenous genes.[5] These mechanisms are known as CRISPRa (activator) and CRISPRi (interference). The potential to modify or correct pathogenic gene mutations makes these strategies attractive for the study and treatment of genetic diseases, including intractable genetic epilepsies.[6,7] It also raises the possibility that fine-tuning gene expression could be utilized for the treatment of diseases without known genetic cause, such as acquired epilepsy.

In their recent study, Colasante and colleagues examined whether CRISPRa-mediated tuning of potassium channel expression could ameliorate disease pathology in a mouse model of temporal lobe epilepsy (TLE).[8] They targeted the promoter region upstream of the gene *Kcna1*, which encodes the $K_v1.1$ voltage-gated potassium channel. $K_v1.1$ activity repolarizes neuronal membranes and regulates action potential firing and synaptic transmission. Overexpression of exogenous

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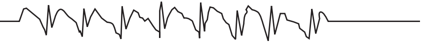

*Kcna1* was previously shown to reduce neuronal excitability and suppress epileptic activity in a rat model.[9]

First, sgRNAs targeting the promoter region of *Kcna1* were tested in combination with dCas9 fused to the transcriptional activator VP160 in mouse P19 cells. The most effective sgRNA was incorporated into a lentivirus construct with dCas9-VP160 and transduced into primary mouse cortical neurons. A construct containing sgRNA targeting LacZ was used as a control throughout the study. *Kcna1* expression was elevated 4- to 6-fold compared to control. Gene expression profiling of transduced neurons revealed no significant off-target effects impacting expression of other genes. Elevated expression of *Kcna1* corresponded with decreased neuronal firing frequency.

The authors then moved their *Kcna1*-dCas9A system into in vivo studies. For efficient transduction of brain neurons, they utilized dual AAV9 viral vectors. Because of the reduced cargo capacity of AAV9, they also switched to the VP64 transcriptional activator. One AAV carried the dCas9-VP64 construct under the control of a doxycycline-inducible promoter. The second AAV carried the *Kcna1*-specific or control sgRNA under the control of the human synapsin promoter, and expression was rendered conditional by inclusion of an inverted flox cassette. The AAV9 viruses were delivered into the hippocampus of *Camk2a-cre* transgenic mice. Thus, the *Kcna1*-dCas9A system could be activated in forebrain neurons by feeding the mice a doxycycline diet. Whole-cell patch clamp analysis in brain slices revealed decreased firing of pyramidal hippocampal neurons.

To test the dual AAV9 *Kcna1*-dCas9A system in a mouse model of TLE, kainic acid was administered to induce a period of status epilepticus followed by development of chronic epilepsy. In this experiment, the sgRNA construct was driven by a *Camk2a* promoter with no floxed cassette. dCas9 activity was controlled by administration of doxycycline. Epileptic mice treated with *Kcna1*-dCas9A exhibited a significant reduction in seizure burden. Interestingly, *Kcna1*-dCas9 had no effect on acute focal seizures induced by pilocarpine. Hippocampal expression of *Kcna1* was elevated less than 1.5-fold in *Kcna1*-dCas9-treated mice. Perhaps higher levels of *Kcna1* expression could confer seizure protection in the acute seizure model. It is also possible that kainic acid and pilocarpine paradigms produce seizures via different underlying mechanisms, and *Kcna1* elevation may not be efficacious across models. Thus, testing of *Kcna1*-dCas9A treatment in a variety of models would be essential in evaluating its potential as a general treatment for epilepsy.

Epileptic mice displayed a cognitive deficit in the object location test, and their performance improved with *Kcna1*-dCas9A treatment. It is not clear whether this improvement was a result of downstream effects of activation of *Kcna1* or a secondary effect from reduction in seizure activity. Transcriptome analysis of hippocampi showed extensive changes in gene expression in epileptic animals that were partially rescued by treatment with *Kcna1*-dsCas9A. More study will be needed to elucidate how gene expression impacts the epileptic phenotype and the efficacy of *Kcna1*-dsCas9A treatment. Theses finding highlight the need to more fully understand the pathology underlying acquired epilepsy and its comorbidities in order to identify additional therapeutic targets that may be amenable to manipulation by CRISPR gene editing technologies.

This study indicates that CRISPR-Cas9-mediated upregulation of *Kcna1* expression could be an effective therapy for acquired epilepsies. Because $K_v$1.1 regulates neuronal excitability, it is appealing as an antiepileptic target with wide potential clinical utility. Ion channel regulation has been the basis for many anticonvulsant drugs. One drug targeting the Kv7 family of potassium channels, retigabine (ezogabine), was approved for the treatment of adult focal seizures. This drug was later withdrawn by the manufacturer due to adverse side effects.[10] It will be important to rigorously determine the safety of targeting Kv1 potassium channels. An advantage of CRISPRa may be reduced off-target effects compared to traditional pharmacologic approaches, which may cause less detrimental side effects. Genetic epilepsies may not be as amenable to this treatment, since it does not correct the underlying cause. Other CRISPR genome editing strategies may be more appropriate in these cases, providing precision treatment of genetic epilepsies.

The major technical obstacle to in vivo use of CRISPR in humans is delivery. The most studied approach is viral delivery using AAVs, adenoviruses, and lentiviruses. The dual AAV9 used in this study may be a good candidate, as AAVs do not provoke a strong immune response. However, AAVs are limited to a relatively small cargo capacity. Development of a smaller dCas9 could allow more efficient packaging for single AAV treatment. To translate CRISPR-mediated therapies into viable treatments for human disease, more work will be needed to find the optimal virus type for low immunogenicity, sufficient packaging, and tissue specificity. As new applications for the CRISPR-Cas system are discovered, including CRISPRa and CRISPRi, precision treatments for genetic and acquired epilepsies may be within reach.

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