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Commentary

Targeting the trunk of multi-root common epilepsy with gene therapy

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The human brain has been called the most complex structure of the known universe, hence the difficulty in understanding it and, as a result, treating neurological diseases. This complexity is determined by the participation of the vast majority of the genome during brain development; thus, there are thousands of individual neurogenetic diseases, most of which include disturbed electrical activity of the brain, presenting as seizures.[1]

As we are now able to identify genetic mutations specific to each patient, we can attempt to replace the affected gene, solving the problem at the root without needing to fully understand all aspects of the intervening neurodevelopmental disorder.[1,2] Because postnatal neurons and astrocytes, for the most part, do not divide, gene replacement is expected to be highly impactful to many patients. However, part of what remains challenging is developing delivery vehicles superior to AAV9, as this commonly used viral vector does not adequately distribute across the brain, and delivering gene therapies at early enough stages, prior to the ravages of the passage of developmental time. In sum, the main challenge of gene therapy is that it has to be individualized to each particular root cause and that present-day vehicles cannot deliver to every single affected cell.[1,2] But what if we move away from the individual roots to the shared "trunk" of certain diseases? Could we apply gene therapy at the trunk, which is more spatially confined and amenable to widespread local distribution of viral vectors, and potentially treat multiple diseases with one gene therapy? In a recent issue of *Molecular Therapy Methods and Clinical Development*, a paper by Beaudoin and colleagues[3] is an important contribution in a series of recent efforts to achieve this.

Mesial temporal lobe epilepsy (mTLE) is the most common form of epilepsy[3] if one classifies epilepsies by the different brain regions where different patients' seizures originate, as we used to do in the pregenetic era. It is now recognized that mTLE is not of one causation but of many, including cases associated with different somatic monogenic diseases.[1,3] The mesial temporal lobe's neural network is simply more prone to seizing than other brain regions, given various similar pathologies. Classically, if we were unable to control seizures with conventional anti-seizure medications, to which mTLE is often resistant,[3] the only option left is to surgically resect the epileptogenic part of the brain. But enough is now known about epileptogenesis that researchers are considering the alternative of intervening locally by using gene therapy approaches, whereby proteins or pathways key to seizure generation or propagation can be locally manipulated or affected in the epileptogenic foci, which is particularly advantageous for focal epilepsies like mTLE.[1–3] Such interventions would have the advantages of comprehensiveness, as locally introduced viral vectors would be able to transduce all cells in that limited specific region, and permanence, by virtue of modification at the genetic level.[1–3]

Of the several approaches to achieve the above, one has been the gene-therapy-based introduction of neuropeptides that regulate neurotransmitter release and neuronal excitability.[4] For instance, galanin, acting on its GalR1/GalR2 receptors, reduces glutamatergic neurotransmission and neuronal excitability and may also be neuroprotective during episodes of prolonged seizures/status epilepticus.[5] AAV-mediated galanin overexpression following injections into the hippocampus was anti-convulsant in rodent models of focal epilepsy.[5]

Somatostatin modulates neurotransmitters, like glutamate and serotonin, that are elevated during seizures, dampens neuronal activity, and enhances GABAergic inhibitory signaling.[4,6] Somatostatin-expressing neurons are abundant in the hippocampus, and AAV5-mediated delivery of this peptide into this structure resulted in significant anti-seizure effects in a rat model of TLE.[6]

Dynorphin is an endogenous opioid peptide associated with neuroprotective and anti-seizure effects via modulation of synaptic release properties and inhibition of excessive excitatory neurotransmission.[4,7] Significant seizure suppression was observed following AAV-mediated hippocampal delivery of preprodynorphin (AAV-pDyn) in a rat model of mTLE.[7]

Finally, the seizure-modulating peptide that has received the most attention is neuropeptide-Y (NPY).[8] For example, viral vectors co-expressing NPY with its inhibitory Y2 receptor, the latter controlled by a CamKIIa promoter to drive expression toward excitatory neurons, were shown to reduce seizure frequency when administered focally into the hippocampus of various TLE rodent models.[8]

A second recent approach is the focal gene-therapy-based introduction of an inhibitory ion channel. Specifically, the *KCNA1* gene, encoding potassium channel Kv1.1, was codon optimized for human expression under the control of a CaMKII promoter. Packaging this construct into lentivirus or AAV2/9 vectors and administrating it focally into the cortex or hippocampus led to a strong reduction of seizures in rat models

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of focal neocortical epilepsy and TLE, respectively.[9] A similar approach has more recently been proven effective in focal cortical dysplasia caused by mTOR (mammalian target of rapamycin) hyperactivity.[10]

In the recent issue of *Molecular Therapy Methods and Clinical Development*, Baudouin and colleagues[3] took the converse approach. Instead of enhancing inhibition, they aimed to reduce excitation. They developed a dual microRNA-containing AAV9 vector, designed to target and knock down *GRIK2* mRNA, encoding the GluK2 protein, which is a glutamate receptor controlling neuronal excitability and transmission. The two microRNAs in the viral vector were controlled by the neuron-specific synapsin promotor, and the viral vector was directly administered into the hippocampus. This resulted in the selective transduction of hippocampal neurons in both the pilocarpine mTLE mouse model and cynomolgus monkeys. Apart from the region- and cell-specific significant reduction of *GRIK2* mRNA and GluK2 proteins lasting up to 6 months in the epileptic mice, their vector also exhibited similar results in successfully transducing cells in human hippocampal slices obtained from patients with medication-resistant mTLE.

One of the most promising outcomes of their approach was the decreased seizure-related activity in both treated epileptic mice and human hippocampal slices following the effective knockdown of GluK2 and by normalizing irregular excitatory activity in the hippocampus. Notably, besides reducing seizures, the viral-vector-treated mice exhibited correlated behavioral improvements in terms of anxiety levels and memory, implying a potential for a range of more comprehensive benefits.[3]

While results of this study are promising, the consequences of a prolonged downregulation of *GRIK2* remain unknown. Compensatory mechanisms could emerge over time that counteract the benefits and compromise the sustained safety and efficacy of this approach. Additionally, while animal models provide valuable preclinical tools, they do not always replicate the pathophysiology of the disease in human patients.

The pathophysiology of mTLE is not uniform, and multiple different approaches may need to be developed to counter each form. The difficulty with this therapeutic personalization will lay in the abilities of epileptologists to distinguish one form from another, which is presently no easy feat, if at all possible. On the other hand, it may turn out that the different forms sufficiently overlap, and an approach such as the one developed by Baudouin and colleagues[3] may apply to most patients. More generally, translation, time, and experience will tell to what extent molecular-level interventions will replace resective or ablative surgery. It is eventually to be expected that molecular precision will supersede scalpel and laser.

AUTHOR CONTRIBUTIONS

M.S. and B.A.M. wrote the manuscript. B.A.M. revised the manuscript.

DECLARATION OF INTERESTS

The authors declare no conflicts of interest.

REFERENCES

1. Klein, P., Kaminski, R.M., Koepp, M., and Löscher, W. (2024). New epilepsy therapies in development. Nat. Rev. Drug Discov. *23*, 682–708.
2. Walker, M.C. (2025). State-of-the-art gene therapy in epilepsy. Curr. Opin. Neurol. *38*, 128–134. https://doi.org/10.1097/WCO.0000000000001349.
3. Baudouin, S.J., Giles, A.R., Pearson, N., Deforges, S., He, C., Boileau, C., Partouche, N., Borta, A., Gautron, J., Wartel, M., et al. (2024). A novel AAV9-dual microRNA-vector targeting GRIK2 in the hippocampus as a treatment for mesial temporal lobe epilepsy. Mol. Ther. Methods Clin., Dev. *32*, 101342.
4. Clynen, E., Swijsen, A., Raijmakers, M., Hoogland, G., and Rigo, J.M. (2014). Neuropeptides as targets for the development of anticonvulsant drugs. Mol. Neurobiol. *50*, 626–646.
5. Wallace, R. (2004). A promising Genetic Approach for the Treatment of Epilepsy. Epilepsy Curr. *4*, 164–166.
6. Zafar, R., King, M.A., and Carney, P.R. (2012). Adeno associated viral vector-mediated expression of somatostatin in rat hippocampus suppresses seizure development. Neurosci. Lett. *509*, 87–91.
7. Agostinho, A.S., Mietzsch, M., Zangrandi, L., Kmiec, I., Mutti, A., Kraus, L., Schwarzer, C., Fidzinski, P., Schneider, U.C., Holtkamp, M., and Heilbronn, R. (2019). Dynorphin-based "release on demand" gene therapy for drug-resistant temporal lobe epilepsy. EMBO Mol. Med. *11*, e9963.
8. Cattaneo, S., Bettegazzi, B., Crippa, L., Asth, L., Regoni, M., Soukupova, M., Zucchini, S., Cantore, A., Codazzi, F., Valtorta, F., and Simonato, M. (2024). Gene therapy for epilepsy targeting neuropeptide Y and its Y2 receptor to dentate gyrus granule cells. EMBO Rep. *25*, 4387–4409.
9. Snowball, A., Chabrol, E., Wykes, R.C., Shekh-Ahmad, T., Cornford, J.H., Lieb, A., Hughes, M.P., Massaro, G., Rahim, A.A., Hashemi, K.S., et al. (2019). Epilepsy gene therapy using an engineered potassium channel. J. Neurosci. *39*, 3159–3169.
10. Almacellas Barbanoj, A., Graham, R.T., Maffei, B., Carpenter, J.C., Leite, M., Hoke, J., Hardjo, F., Scott-Solache, J., Chimonides, C., Schorge, S., et al. (2024). Anti-seizure gene therapy for focal cortical dysplasia. Brain *147*, 542–553.