Development of a novel AAV9 gene therapy for the treatment of temporal lobe epilepsy using animal model and human organotypic slices

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INTRODUCTION

Temporal lobe epilepsy (TLE) is a common form of epilepsy characterized by recurrent seizures generated in the limbic system, particularly in the hippocampus. Patients with TLE are often resistant to currently available anti-seizure medications. Recurrent mossy fiber sprouting from dentate granule cells (DGCs) is a consistent anatomical hallmark in the hippocampus of TLE patients and is recapitulated in the mouse pilocarpine model of TLE used in our studies. This synaptic reorganization creates an aberrant excitatory network between DGCs that operates via ectopic expression of GluK2/GluK5-containing kainate receptors and plays a central role in the generation of seizure activity. With our study, we identified different strategies to decrease GluK2 protein expression using specific synthetic miRNAs. We developed an AAV9-miRNA vector optimized for full AAV packaging, generating a construct with two miRNAs sequences. The miRNA expression was controlled by the neuron specific human synapsin1 promoter (hSyn1). Treatment of mouse cortical neurons with the optimized AAV9 construct led to a significant decrease in GluK2 protein expression. Direct delivery of vector constructs expressing anti-GRIK2 miRNAs into the hippocampus of pilocarpine-induced TLE mice led to a marked reduction in the aberrant behaviour and frequency of epileptiform discharges (EDs). Similarly, treatment of organotypic hippocampal slices from refractory TLE patients who underwent resection surgery led to a significant decrease in the expression of Gluk2 and decrease in the frequency of EDs. Our developed AAV9 gene therapy product showed good manufacturing properties with the ability to reduce EDs in TLE mice and tissue from patients with TLE.

OBJECTIVES

We developed a gene therapy approach to reduce epileptiform discharges in an animal model of TLE and in hippocampal organotypic slices from patients with TLE. The aim of the study was to:

1. **Establish a proof of concept for a gene therapy** approach using an AAV9 coding for a single miRNA targeting GRIK2.
2. **Optimize the AAV9 construct** for GRIK2 knockdown potential and manufacturability.
3. **Determine the efficacy of the optimized construct** on epileptiform activities in mouse model and human organotypic slices.

METHODS

In vitro method

The effect of the different constructs on GRIK2 mRNA and GluK2 protein expression levels were assessed using cortical neuron cultures from mice.

Mouse model for TLE

The mouse pilocarpine model was used as described in Vigier 2021. After 2 months, the viral vector was administered at two locations in both hippocampi. Locomotor activity and number of epileptiform discharges per day were used as efficacy readouts.

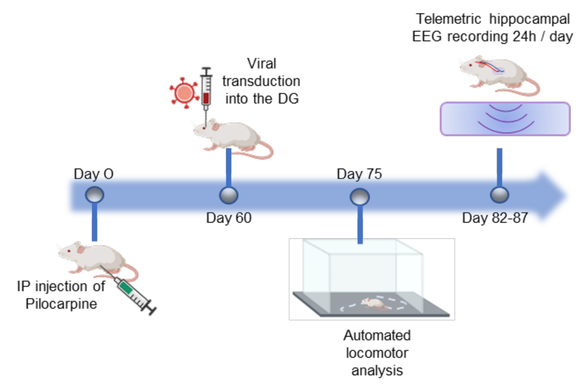


Figure 1 – Experimental design of the pilocarpine mouse model study.

Human organotypic slices

Acute and organotypic slices were prepared from surgical resections of the hippocampus from 6 patients (11 – 60 years old) diagnosed with drug-resistant TLE. The slices were treated after one day in vitro (DIV1). Electrophysiological recordings were performed between DIV10 and DIV14.

RESULTS

Localisation in the hippocampus

When under the control of human synapsing 1 promoter, GFP expression was restricted to neurons whereas when under the control of CAG it was expressed in various neuronal population.

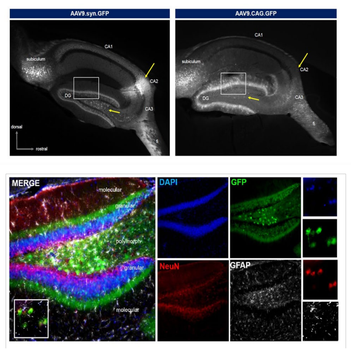


Figure 2 – Localisation of GFP after intra-hippocampal injection of AAV9 containing an hSyn1 or a CAG promoter. *A. Lower magnification showing the distribution of GFP across the mouse hippocampus. B Higher magnification showed that under the hSyn1 promoter, GFP expression co-localizes with the neuronal marker NeuN and not with the glial marker GFAP. Dapi stains the nucleus.*

Injection of AAV9 expressing a single miRNA against GRIK2 leads to a significant decrease in pilocarpine treated mice hyperactivity and epileptiform discharges recorded with electroencephalogram (EEG). The treatment of organotypic hippocampal slices from patient with TLE with miRNA targeting GRIK2 leads to a significant decrease in the number of ED

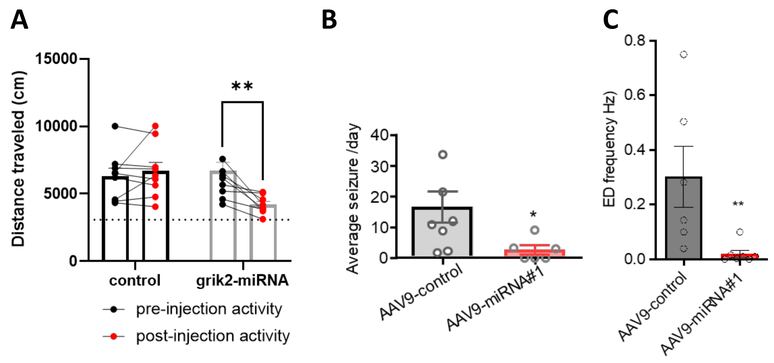
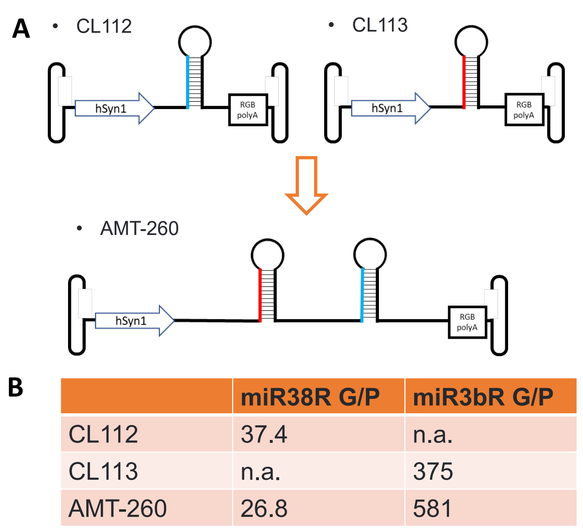


Figure 3 – Effect of GRIK2 knockdown in a mouse model of TLE and in hippocampal organotypic slices from patients with TLE. *A. Locomotion behavior in the open field B. Epileptiform activities quantified in pilocarpine mice. C Quantification of ED frequency in human organotypic slices. Average ± s.e.m. * P < 0.05, ** P < 0.01.*

Construct optimization

We designed a construct expressing two antisense RNA sequences, miR38R and miR3bR, targeting different parts of GRIK2 sequence. These sequences were included in a single AAV9 construct and placed under the control of the hSyn1 promoter. The construct was then optimized to express significantly more guide than passenger strand.



B

	miR38R G/P	miR3bR G/P
CL112	37.4	n.a.
CL113	n.a.	375
AMT-260	26.8	581

Figure 4 – Design of a dual miRNA AAV9 construct *A. Two distinct antisense RNA sequences are expressed in an AAV9 under the control of hSyn1. B Guide to passenger strand ratio (G/P) for each of the constructs.*

The combination of the miR38R and miR3bR in one AAV9 under the control of a single promoter leads to a potentiation of their expression in mouse cortical neurons. Altogether, AMT-260 showed a higher potential in reducing GRIK2 mRNA expression compared to a single antisense RNA sequence approach.

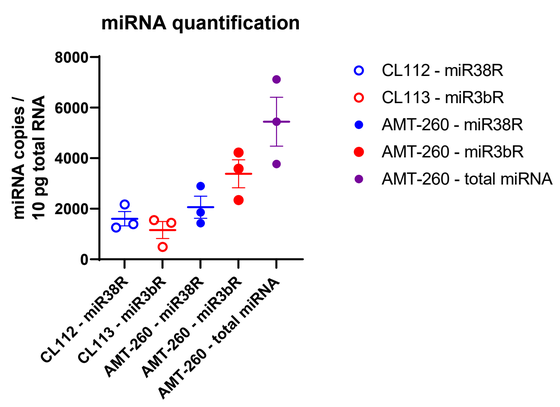


Figure 5 – Quantification of antisense RNA expression. *Average ± s.e.m.*

The treatment of mouse cortical neurons with AMT-260 led to a significant decrease of GRIK2 mRNA. The treatment of mouse cortical neurons with AAV9 expressing a single antisense RNA sequence did not significantly affect GRIK2 mRNA expression.

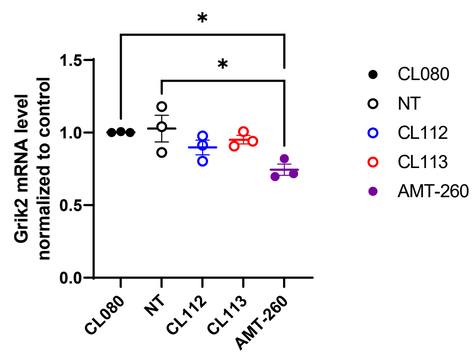


Figure 6 – Quantification of GRIK2 mRNA expression in mouse cortical neurons. *Average ± s.e.m. * P < 0.05.*

Injection of AMT-260 in the hippocampus of mice treated with pilocarpine leads to a significant decrease of hyperactivity. Injection of AAV9 expressing a single antisense RNA sequence did not significantly affect the hyperactivity of pilocarpine treated mice.

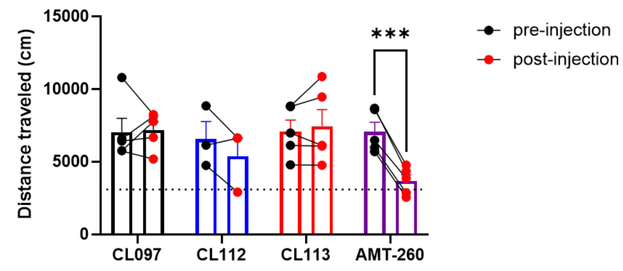


Figure 7 – Effect of AMT-260 treatment compared to AAV9 single antisense RNA sequence on the hyperactivity in a mouse model of TLE. *Average ± s.e.m. *** P < 0.001.*

Efficacy of the optimized construct

Intra-hippocampal injection of increasing doses of AMT-260 in pilocarpine treated mice correlated with an increased expression miR38R and miR3bR.

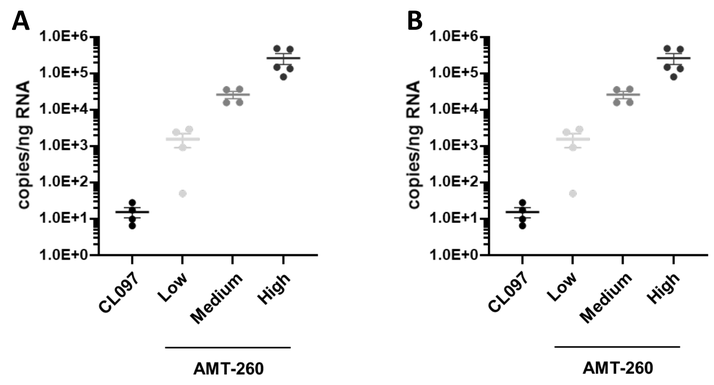


Figure 8 – Quantification of antisense RNA expression in the hippocampus of pilocarpine treated mice. *A) miR38R and B) miR3bR. Average ± s.e.m.*

Injection of high dose of AMT-260 led to a decrease of activity in pilocarpine treated mice, to levels of activity observed in wild type mice. Lower doses of AMT-260 did not show significant effects.

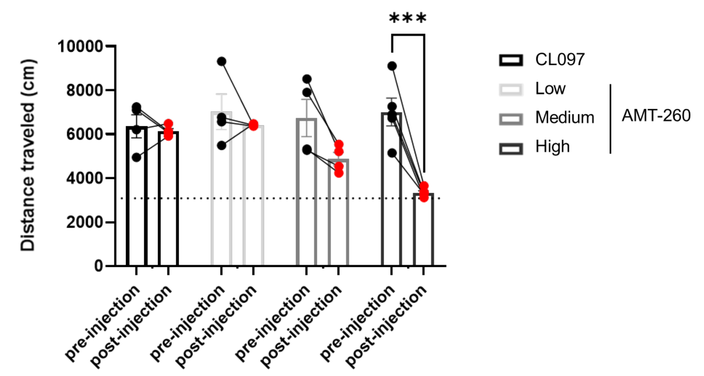


Figure 9 – Effect of increasing doses of AMT-260 on the locomotion of pilocarpine treated mice. *The dotted line represent the average distance travelled by wild type mice. Average ± s.e.m. *** P < 0.001.*

Injection of the high dose of AMT-260 led to a decrease in the number of ED in pilocarpine mice. Treatment of human organotypic slices from patients with TLE decreased significantly the number of ED.

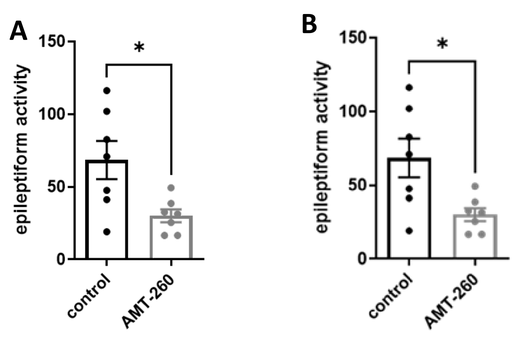


Figure 9 – Effect of AMT-260 on the number of ED. *A) pilocarpine treated mice and B) organotypic slices from patients with TLE. Average ± s.e.m. * P < 0.05.*

CONCLUSIONS

Overall our data showed that lowering GRIK2 mRNA using an AAV9 vector alleviated the phenotypes of a TLE mouse model and of organotypic slices from patients with TLE.

More specifically, we demonstrated that:

- Two miRNA approach to knock-down GRIK2 decrease the hyperactivity and the EDs in a mouse model of TLE.
- Two miRNA approach to knock-down GRIK2 decrease the EDs in organotypic slices from patients with TLE.
- The optimization of the AAV9 construct provided a product a vector with increased potential to reduce GRIK2 miRNA without affecting its biological effect.

These studies provide solid evidence that AMT-260 is efficacious in both a mouse model for TLE and in tissue from patients. AMT-260 is therefore a promising candidate for gene therapy for TLE.

REFERENCES

1. Vigier et la. (2021) *Neurobiol Dis. 161:105547.*

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