MicroRNA-mediated modulation of electrographic activity in a Cntnap2 mouse model of epilepsy

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What is *Cntnap2* and why study epilepsy?

- *Cntnap2* encodes contactin-associated protein-like 2 (CASPR2), a protein that plays a vital role in neuron-glia interaction and action potential propagation (Poliak et al., 2000)

- CNTNAP2 mutations in humans are associated with epilepsy and autism (Friedman et al., 2008), and display symptoms including seizures and intellectual disability (Strauss et al., 2006)

- 5 million people are diagnosed with epilepsy every year (WHO, 2019), and annual costs of epilepsy on our society are about $28 billion (Prescott et al., 2020)

**Mouse model:**

- *Cntnap2* knockout mice are a vital tool to study this gene’s association with epilepsy, as first reported by Peñagarikano et al., 2011

  - The *Cntnap2* model of epilepsy displays seizures, abnormal EEG patterns, and neuronal migration (Thomas et al., 2016)
What are microRNAs (miRNA)?

- MiRNAs are non-coding single stranded RNAs which regulate post-transcriptional expression of mRNA and can be targeted to regulate gene expression through translational inhibition.

- MiRNAs play a vital role in regulating seizures in epilepsy.
  - Based on previous work in the Gross Lab and other studies, two notable miRNAs are vital for role in regulating neuronal network and providing neuroprotection:
    - miR-324-5p (Gross et al., 2016); (Tiwari et al., 2019)
    - miR-218-5p (Kaalund et al., 2014)

- Antagomir Treatments as a therapy for epilepsy
  - Antagomirs are antisense oligonucleotide treatments targeted to miRNA sequences which can regulate the expression of specific proteins by preventing the binding of miRNA to their mRNA targets.
  - They have been shown to have therapeutic effects in the regulation of epilepsy (Gross et al., 2016); (Tiwari et al., 2019).
Aim

• Research Question:
  – Does the inhibition of candidate microRNAs miR-218-5p and miR-324-5p using antagomirs affect seizure susceptibility in the Cntnap2 knockout mouse model of epilepsy?

• Hypothesis:
  – In vivo antagomir inhibition of miR-218-5p and miR-324-5p will regulate seizure susceptibility and affect electrographic dynamics in Cntnap2 knockout mouse model of epilepsy.
Methods

• Mice
  – Mice were first genotyped
  – They were then classified by gender and age
    • Both male and female mice were used at two age points
      • Age points: younger (4-6 months) or older (12-16 months)
    – This allowed us to explore age and gender specific seizure development

• Transmitter Implantation
  – Electrodes were surgically implanted in mice using a wireless telemetric device, and video EEG was monitored
Antagomir Injection

- Antagomirs for miR-324-5p, miR-218-5p, or scrambled (control) were injected intracerebroventricularly by (ICV), and electrodes were implanted in mice in order to obtain a baseline cortical EEG measurement in young and old mice.
EEG Analysis

- EEG was recorded using a DSI wireless telemetry system and analyzed using Neuroscore software.

- Artifacts were manually excluded from analysis (A, electrical noise).
- Seizures were manually scored using video EEG monitoring (B).

- EEG waveform voltage was recorded, and EEG power analysis was conducted after data was exported in 10 second epochs.

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<table>
<thead>
<tr>
<th>Frequency Range</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma: 30-100Hz</td>
<td>Peak performance, flow</td>
</tr>
<tr>
<td>Beta: 12-30Hz</td>
<td>Alert, normal alert or wakefulness</td>
</tr>
<tr>
<td>Alpha: 8-12Hz</td>
<td>Relaxed, calm, lucid, not thinking</td>
</tr>
<tr>
<td>Theta: 4-7Hz</td>
<td>Deep relaxation and meditation, mental imagery</td>
</tr>
<tr>
<td>Delta: .1-4Hz</td>
<td>Deep, dreamless sleep</td>
</tr>
</tbody>
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A. Non-epileptiform spikes
B. Epileptiform spikes: number indicates the seizure stage

Tse et al 2014
Results
Spontaneous recurring seizure (SRS) onset in *Cntnap2* KO mice

EEG and video were monitored in younger and older mice to **confirm** the onset of seizures.

**A**: In younger mice, spikes were observed in EEG but no seizures.

**B**: In older mice, SRS were observed (circled).

Seizures occur at an older age in *Cntnap2* KO mice (12-16 months).
Effect of antagomir treatments on seizure frequency and duration in older Cntnap2 KO mice

Seizures were manually scored and classified based on their duration (A-F)

One way ANOVA (Graph pad)

- a-miR-SCR: 7
- a-miR-218-5p: 5
- a-miR-324-5p: 8

A: p=.0184
B: p=0.438
C: p=0.354
D: p=0.109
E: p=0.229
F: p=0.366

miR-324-5p inhibition decreased seizure frequency and miR-218-5p increased seizure frequency across all seizure duration groups
Effect of antagomir treatment on behavioral and non-behavioral seizure frequency in older Cntnap2 KO mice

Seizures were also classified based on whether they were behavioral (confirmed with video) or non-behavioral (electrographic with no behavior).

One way ANOVA (Graph pad)
- a-miR-SCR: 7
- a-miR-218-5p: 5
- a-miR-324-5p: 8
- Behavioral: p=0.298
- Non-behavioral: p=0.298

Again, a-miR-324-5p treatment reduced seizure frequency and a-miR-218-5p treatment increased frequency for both groups.
Effect of antagomir treatments on EEG power in older Cntnap2 KO mice

- EEG recording was exported and analyzed on Neuroscore

- Average EEG power of four different waveforms, (δ(A), θ(B), β(C), and γ(D)), were analyzed and compared between treatment groups

One way ANOVA (Graph pad)

a-miR-SCR: 3
a-miR-218-5p: 2
a-miR-324-5p: 3

A: p=0.301
B: p=0.157
C: p=0.250
D: p=0.725

No significant differences in EEG power for any waveform were found between the treatment groups.
Effect of antagonir treatments on epileptiform non-seizure EEG spikes in older Cntnap2 KO mice

- Spike analysis was conducted to determine how many epileptiform non-seizure related spikes were found in the EEG

- Spikes indicate an increase in hyperactivity in the neuronal network

One way ANOVA (Graph pad)

a-miR-SCR: 3
a-miR-218-5p: 2
a-miR-324-5p: 3

p=0.469

miR-324-5p inhibition had a trend to increase epileptiform EEG spikes
Effect of antagomir treatment on kainic acid-induced seizure onset latency in younger Cntnap2 KO mice

- Young mice underwent kainic acid treatment after 10 days to induce seizures
- Seizure onset time was measured and compared between groups
- Antagomir treatment for miR-324-5p significantly increases latency to KA-seizure onset while it is decreased with treatment for miR-218-5p
Effect of antagomir treatments on EEG power of younger *Cntnap2* KO mice

- Power analysis, following the same procedure as with older mice, was performed to assess EEG power for:
  - A: Delta wave
  - B: Theta wave
  - C: Beta wave
  - D: Gamma wave

A trend towards significance ($p=.0594$) was observed of the average EEG power of Delta waves in a-miR-324-5p mice compared to other treatments, but not in any other waveforms.
Effect of antagomir treatment on spikes of younger *Cntnap2* KO mice

- Spike analysis, following the same procedure as with older mice, was performed on younger mice
- This was another way for us to explore hyperactivity of the neuronal network

A trend was observed for an increase in spikes in mice treated with a-miR-324-5p that can be further investigated
Ongoing Tissue Analysis

• Mouse brains were collected to analyze the effect of treatment on **cell death**, **neuronal migration** and **gliosis** in the brain

• **Ongoing immunostaining:**
  – NeuN (neuronal, A)
  – GFAP (Astrocytes)
Conclusions

• ICV injection of antagonim for miR-324-5p in Cntnap2 KO mice resulted in a delayed onset of seizures (younger mice) as well a decrease in seizure severity (older mice)

• Cntnap2 KO mice treated with miR-218-5p antagonim injection displayed an increase in seizure severity and decrease in seizure onset latency

• Preliminary EEG waveform power and spike analyses did not find any significant differences between EEG power and epileptiform spikes between treatment groups.

These results suggest that both miR-324-5p and miR-218-5p differentially regulate seizures in Cntnap2 KO mice and could potentially be important for studying the mechanism of epilepsy
Future Aims

• Increase sample size for EEG power analysis of younger and older mice

• Explore localization of effects by conducting immunohistochemical tissue analysis of hippocampus and cortex

• Expand EEG power and spike analysis to include sleep-wake patterns in EEG recordings
References


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If you have any questions, feel free to contact me at: mukherry@mail.uc.edu!