Veterinary Research Scholars Program
University of Missouri

Innovative RNA-Targeting CRISPR Therapy for *SYT1*-Associated Neurodevelopmental Disorder

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ABSTRACT

Baker-Gordon syndrome (BAGOS) is a rare neurodevelopmental disorder in humans. BAGOS is caused by heterozygous mutations in the *SYT1* gene resulting in production of a malfunctioning SYT1 protein leading to improper neurotransmitter release at the synapse. There is currently no cure for this disease. Our innovative project uses the CRISPR-Cas13 system to target mutant RNA transcripts from the defective *SYT1* gene. Cas13 is a component of the CRISPR system that targets and degrades mRNA. However, it is unknown if Cas13 can be utilized to target a specific patient-derived mutation that causes BAGOS. We hypothesize that gRNAs for the CRISPR-Cas13 system can be designed specific enough to effectively target mutant *SYT1* mRNA transcripts while leaving wild type (WT) *SYT1* mRNA transcripts intact. Our initial experiment involves the design of 8 gRNAs tailored to the mutant *SYT1* D366E mRNA transcript. Out of these gRNAs, 4 unique gRNA sequences will be used in conjunction with both Cas13a and Cas13d subtypes. These complexes will be systematically combined with various ratios of synthetic mutant *SYT1* D366E RNA and WT *SYT1* RNA templates to evaluate the specificity of each complex. Two gRNAs targeting a region of mRNA applicable to both the WT and mutant templates will serve as positive controls. Non-targeted Cas13 complexes will be used as negative controls. We will evaluate targeting efficiency of each combination individually using gel electrophoresis alongside naïve synthetic WT and mutant mRNA transcripts. We expect that both subtypes of Cas13 will be able to effectively target mutant mRNA while leaving the WT intact. This discovery will provide feasibility that Cas13 may be a future therapeutic option for patients with BAGOS.

BACKGROUND

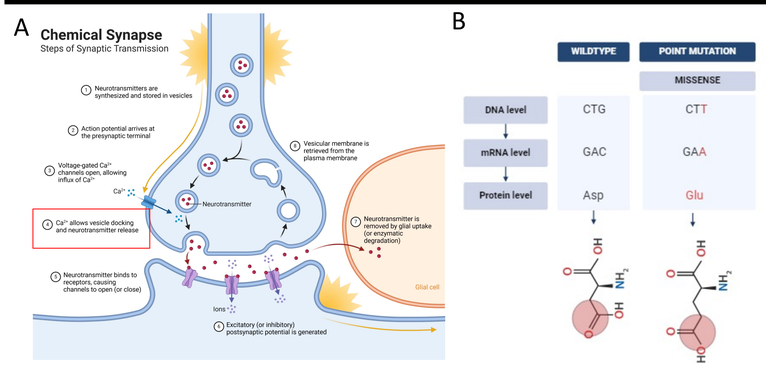

Figure 1. The synaptotagmin-1 protein present in the membrane of synaptic vesicles binds calcium to facilitate neurotransmitter exocytosis and neuronal action potential propagation. Affecting the 4th step of synaptic transmission (red box) **(A).** The *SYT1* D366E human variant mutation causes an aspartic acid to glutamic acid substitution that affects the calcium binding (CB2) domain **(B).**

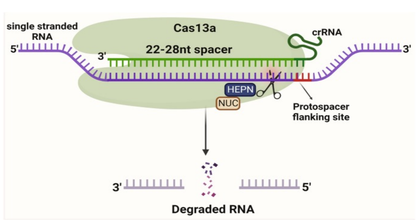

Figure 2. CRISPR-Cas13 complex targets and degrades mRNA using unique gRNA.

HYPOTHESIS

We hypothesize that gRNAs for the CRISPR-Cas13 system can be designed specific enough to effectively target mutant *SYT1* mRNA transcripts while leaving wild type (WT) *SYT1* mRNA transcripts intact.

METHODS

Design gRNA for Cas13a and Cas13d that will target mutant *SYT1* mRNA

- Using Cas13 gRNA tool, 3 gRNA sequences specific to D366E were used to be used in conjunction with both Cas13a and Cas13d subtypes
- Two gRNAs targeting a region of mRNA applicable to both the WT and mutant template served as positive controls
- Non-targeted Cas13 complexes were used as negative controls

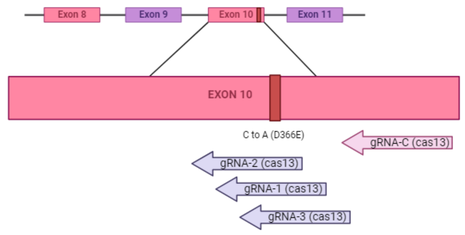

Figure 3. Three gRNAs were designed to target the point mutation in exon 10 of the D366E mRNA template. One gRNA targets a region of exon 10 applicable to both WT and mutant mRNA templates.

Test different Cas13 protein subtypes and gRNAs *in vitro* for targeting of D366E mutant *SYT1* mRNA transcript

- gRNAs were combined with Cas13 proteins to create the CRISPR-Cas13 complexes
- CRISPR-Cas13 complexes were combined with WT *SYT1* mRNA and mutant *STY1* mRNA
- Gel electrophoresis was used to evaluate targeting efficiency

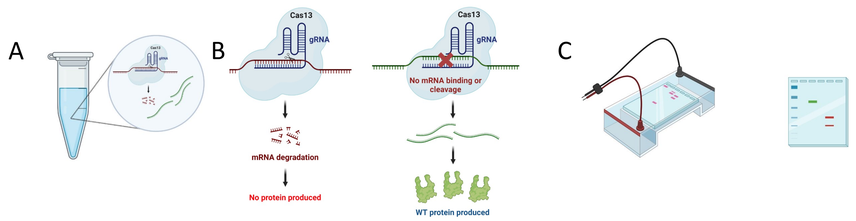

Figure 4. Combining the CRISPR-Cas13 complexes with either synthetic WT or mutant mRNA templates **(A)**. CRISPR-Cas13 complexes should be specific enough to target mutant mRNA and not WT mRNA templates **(B)**. Gel electrophoresis was used for evaluation **(C)**.

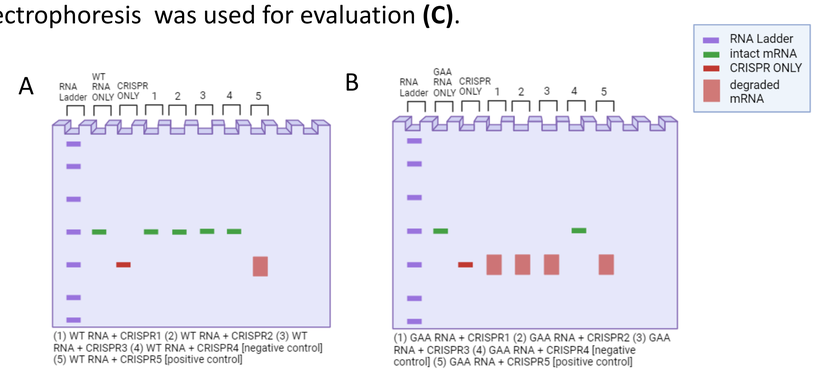

Figure 5. Expected results in gel shift assay for WT mRNA when combined with various CRISPR-Cas13 complexes **(A)**. Expected results in gel shift assay for mutant mRNA when combined with various CRISPR-Cas13 complexes **(B)**.

RESULTS

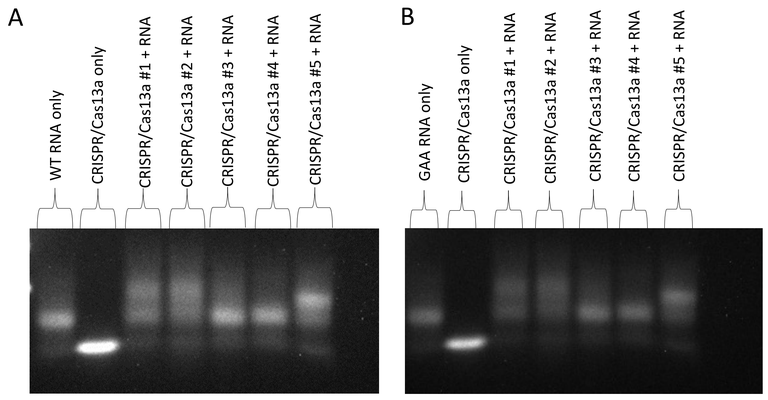

Figure 6. Gel assay results using WT *SYT1* RNA template **(A)**. Or D366E *SYT1* RNA template **(B)**. A 2% TBE agarose gel stained with SYBR Gold was used. From left to right consisted of: 1.0 µg RNA template only, 0.5 µg CRSIPR/Cas13a only, 1.0 µg RNA + 0.5 CRISPR/Cas13a #1, 1.0 µg RNA + 0.5 CRISPR/Cas13a #2, 1.0 µg RNA + 0.5 CRISPR/Cas13a #3, 1.0 µg RNA + 0.5 untargeted CRISPR/Cas13a (- control), 1.0 µg RNA + 0.5 CRISPR/Cas13a #5 (+ control).

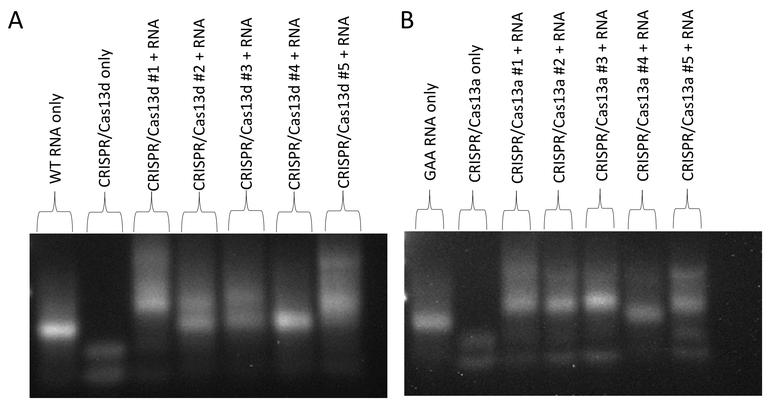

Figure 7. Gel assay results using WT *SYT1* RNA template **(A)**. Or D366E *SYT1* RNA template **(B)**. A 2% TBE agarose gel stained with SYBR Gold was used. From left to right consisted of: 1.0 µg RNA template only, 0.5 µg CRISPR/Cas13d only, 1.0 µg RNA + 0.5 CRISPR/Cas13d #1, 1.0 µg RNA + 0.5 CRISPR/Cas13d #2, 1.0 µg RNA + 0.5 CRISPR/Cas13d #3, 1.0 µg RNA + 0.5 untargeted CRISPR/Cas13d (- control), 1.0 µg RNA + 0.5 CRISPR/Cas13d #5 (+ control).

CONCLUSIONS

- Preliminary results indicate Cas13d appeared to be more active than Cas13a for desired results
- Cas13a showed less specificity and targeted both wildtype and mutant RNA templates equally
- Cas13d predominantly targeted mutant RNA template while leaving majority of WT RNA template intact, with CRISPR/Cas13 complex #2 and #3 showing the most promise

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