Angelman Syndrome is a neurological disorder associated with severe mental and motor impairments[1]. One gene associated with the syndrome is the RNA processing gene, *SNRPN*, which is normally expressed at high levels in neurons[2]. Neuronal dysfunction is often attributed to defects in RNA processing[3]. In addition, RNA processing defects can lead to neurodegeneration associated with epilepsy[4]. It is known that patients with specific SNRPN mutations have epileptic phenotypes, yet the molecular mechanism by which this occurs is unknown[5].

My **primary goal** is to determine the role of SNRPN in regulating RNA processing during normal neuron function. To accomplish this, I will use the mouse SNRPN homolog, Snrpn. I will be using mice because Snrpn mutants exhibit epilepsy, which can be quantified with an EEG test that can be compared to the wild type EEG results.

My **hypothesis** is that specific mutations in SNRPN lead to defects in RNA processing in motor neurons, causing epilepsy. My **long-term goal** is to determine if defects in SNRPN RNA processing is responsible for other phenotypic effects in patients with Angelman Syndrome.

**Aim 1:** Determine which SNRPN domains are necessary for proper RNA processing in neurons.

**Approach**: I will use the SMART database to identify conserved domains within SNRPN. I will use CRISPR to knockout a single domain, and conduct an EEG test. These results will be compared to wild type to identify mutants that exhibit epilepsy. This will be repeated with each domain.

**Rationale:** Because single domains will be evaluated in each trial, the phenotypic differences compared to wild type can be easily identified. The mutants that exhibit epilepsy will indicate that the corresponding domain is critical to proper neuronal function.

**Hypothesis:** The Sm domain of SNRPN is required for RNA binding and the LC domains will be required for associating with the spliceosome complex, therefore inhibiting the function of either of these domains will prevent proper neuronal RNA processing from occurring, leading to epilepsy.

**Aim 2:** Determine if domain mutants of *SNRPN* lead to differential expression of neuronal genes involved in RNA processing.

**Approach:** Domain mutants will be created using CRISPR and neuronal gene expression of each mutant will be individually evaluated. I will use RNA-seq analyze the expression of neuronal genes between wild type and SNRPN domain mutants. Mutants with differential expression will be examined for epilepsy to determine if alternate gene expression disrupts neuronal function. I will then use PANTHER to determine what gene ontology groups are differentially expressed in epileptic mice.

**Rationale:** If differential expression is evident in mutants compared to wildtype, it may indicate that *SNRPN* mutations are responsible. Furthermore, if domain mutants are enriched for alternate transcripts are also epileptic, the gene ontology groups with differential expression may also be responsible for neuron function.

**Hypothesis:** Sm domain mutants will not be able to bind RNA and LC domain mutants will not be able to properly associate with the spliceosome, therefore these mutants will not be able to process neuronal transcripts and result in epilepsy. Epileptic mice will alternate expression for genes involved in splicing, locomotion, and stimulus response compared to wildtype.

**Aim 3:** Identify novel protein interactors of SNRPN that are critical for RNA processing in motor neurons.

**Approach:** I will conduct AP-MS to isolate and identify SNRPN protein interactors through co-immunoprecipitation in neurons of wild type mice, and mutant mice for each specific domain. I will compare the protein interactors among all samples and isolate the proteins that can interact with wild type mice but not the domain mutants. I will then identify the interactors by mass spectrometry. I will subsequently use CRISPR to knockout these novel interactors and perform an EEG epilepsy test to determine if the protein is required for neuronal function.

**Rationale:** By identifying novel protein interactors of SNRPN, I can determine what other associates of SNRPN are relevant for neuronal function.

**Hypothesis:** SNRPN LC domains will be involved in interactions with spliceosome regulatory and complex proteins required for neuronal RNA processing. CRISPR knockouts of these proteins will result in epileptic mice. Sm domain mutants will still be able to associate with interactors comparable to wildtype because the Sm domain will be required for RNA binding rather than protein-protein interactions.

**[1]** Angelman Syndrome: Genetics Home Reference

[<https://ghr.nlm.nih.gov/condition/angelman-syndrome>](https://ghr.nlm.nih.gov/condition/angelman-syndrome)

**[2]** Li, H., Zhao, P., Xu, Q., Shan, S., Hu, C., Qiu, Z., & Xu, X. (2016). The autism-related gene SNRPN regulates cortical and spine development via controlling nuclear receptor Nr4a1. *Scientific Reports,* *6*, 29878. doi:10.1038/srep29878

<https://www.ncbi.nlm.nih.gov/pubmed/1533223>

**[3]** Neuropathology: An illustrated interactive course for medical students and residents [<http://neuropathology-web.org/chapter9/chapter9hAtaxia.html>](https://academic.oup.com/hmg/article/8/2/337/585544/The-Chromosome-15-Imprinting-Centre-IC-Region-Has)

**[4]** Gallo, J. . (2005). The role of RNA and RNA processing in Neurodegeneration. *Journal of Neuroscience*, *25*(45), 10372–10375. doi:10.1523/jneurosci.3453-05.2005

< http://www.jneurosci.org/content/25/45/10372 >

**[5]** Battaglia, A., Gurrieri, F., Bertini, E., Bellacosa, A., Pomponi, M. G., Paravatou-Petsotas, M., . . . Neri, G. (1997). The inv dup(15) syndrome: A clinically recognizable syndrome with altered behavior, mental retardation, and epilepsy. *Neurology,* *48*(4), 1081-1086. doi:10.1212/wnl.48.4.1081

<https://www.ncbi.nlm.nih.gov/pubmed/9109904>

**[6]** Johnstone, K. A. (2005). A human imprinting centre demonstrates conserved acquisition but diverged maintenance of imprinting in a mouse model for Angelman syndrome imprinting defects. Human Molecular Genetics, 15(3), 393-404. doi:10.1093/hmg/ddi456

[<https://www.ncbi.nlm.nih.gov/pubmed/16368707?dopt=Abstract>](https://www.ncbi.nlm.nih.gov/pubmed/16368707?dopt=Abstract)