Worldwide, Autism Spectrum Disorder (ASD) affects approximately 1 in 59 children and is four times more prevalent in males [1, 2]. It can lead to sensory difficulties, impaired social behaviors and in some cases intellectual disability [3]. ASD cases are composed of heterogenous mutations creating a variety of phenotypes, yet SYNGAP1 has been found to be one of the most frequently mutated genes linked with ASD. Molecularly, it plays a regulatory role within neuronal phosphorylation pathways and AMPAR localization to the cell membrane [4,5]. Correlations between SYNGAP1 mutations and disruptions of synaptic plasticity, a characteristic of intellectual disabilities, along with abnormal brain functions, typical for sensory deficits were observed; however, *it is unclear if SYNGAP1 plays a role in the most common symptom: impaired social behavior* [6,7].

My **objective** is to determine the role of SYNGAP1 in social behavior and how mutations in SYNGAP1 phosphorylation sites differentially effect social behavior phenotypes in males and females. Danio rerio will be utilized as a model organism due to their similar brain structures to that of humans and the ability to screen for social behavior [8,9]. I **hypothesize** SYNGAP1 phosphorylation will play a vital role in social behavior pathways and, when mutated, will provide insights behind the gender differences seen in ASD [10]. My **long-term goal** is to provide some new understanding of Autism’s large range of disorders, and ultimately a possible target for treating the most common and often difficult symptom, social impairment.

**Aim 1:** **Identify conserved phosphorylation sites necessary for social behavior in SYNGAP1**

**Rationale:** Mutating a serine conserved in all species will be necessary when observing SYNGAP1’s function outside of the brain, and further it will act as a secondary control when specifically observing SYNGAP1’s function in the brain. Mutating a serine that is conserved in neuron possessing species only will be necessary for observing social behavior deficits.

**Approach:** Utilize NCBI Blast to determine homologs for the SYNGAP1 gene, align the sequences using ClustalOMEGA and identify conserved phosphorylation sites with NetPhos. Next, use CRISPR/Cas9 to induce mutations in serine 839, a site conserved across all species, and serine 861, a site conserved by neuron possessing species, in both males and females producing a total of four transgenic lines [11]. Finally, perform a shoaling screen to identify social behavior mutants.

**Hypothesis:** Social behavior impairments will be seen in all mutants, but mutations in the phosphorylation site conserved in nerve species will show a larger deficit. Males will see a more severe deficit than females.

**Aim 2:** **Profile differentially expressed genes associated with social behavior mutants**

**Rationale:** Identifying genes that are differentially expressed with social behavior mutants will provide more insight into SYNGAP1’s interaction network within the brain.

**Approach:** Conduct RNA-seq on brain tissue from male wildtype fish and both male mutant varieties. Wildtype fish will be compared to mutants and results will be sorted using gene ontology terminology.

**Hypothesis:** Serine 839 mutants will show similar gene expression to wildtype fish, but serine 861 mutants will see an increase in Ras, Rap and AMPAR gene expression and a decrease in kinases such as MAPK and CAMK II.

**Aim 3: Identify SYNGAP1 interacting proteins associated with social behavior that differ by gender**

**Rationale:** Will determine if males have a higher ASD prevalence due to unique SYNGAP1 protein interactions in non-brain structures. Identifying these proteins will showcase any gender differences.

**Approach:** Utilize iTRAQ and mass spec on the uterus and teste tissue from both wildtype fish and serine 839 mutant fish. Use CRISPR/Cas9 to mutate proteins identified in the wildtype fish only and are unique between males and females. Expose new mutants to a shoaling screen to recognize correlation between new proteins and social behavior impairments.

**Hypothesis:** Kinases will interact with wildtype SYNGAP1, but not mutant. Males will have a greater number of proteins identified than females, and a majority of newly associated proteins will be kinases or signal molecules involved in phosphorylation.

ASD effects a large population yet remains widely misunderstood due to its wide range of phenotypes and associated genes. Taking an in depth look at SYNGAP1 will not only help us better understand the molecular pathways inducing social behavior impairments, but also a greater knowledge base of the network underlying this highly prevalent disorder.

References

1. *Data & Statistics on Autism Spectrum Disorder | CDC*. Centers for Disease Control and Prevention, Jan. 2014.
2. Baron-Cohen S, Lombardo M, Auyeung B, et al. Why are Autism Spectrum Conditions More Prevalent in Males?. *PLOS Biology*. 2011.
3. Neale, Benjamin M., et al. (2012). Patterns and Rates of Exonic De Novo Mutations in Autism Spectrum Disorders. *Nature News*.
4. O’Roak, B. J. et al. (2014). Recurrent de novo mutations implicate novel genes underlying simplex autism risk. *Nature Communications.* **5**, 5595.
5. Jeyabalan, Nallathambi and James P Clement. SYNGAP1: Mind the Gap. *Frontiers in cellular neuroscience* vol. 10 32. 15 Feb. 2016, doi:10.3389/fncel.2016.00032
6. Clement, J. P., Aceti, M., Creson, T. K., et al. (2012). Pathogenic SYNGAP1 mutations impair cognitive development by disrupting maturation of dendritic spine synapses. *Cell*, *151*(4), 709-723.
7. Michaelson S.D., Ozkan E.D., Aceti M, et al. (2018). SYNGAP1 heterozygosity disrupts sensory processing by reducing touch-related activity within somatosensory cortex circuits. *Nature Neuroscience,* 21, 1-13.
8. Kabashi, Edor., et al. Zebrafish Models for the Functional Genomics of Neurogenetic Disorders. *Biochim Biophys acta*, Molecular Basis of Disease, Mar. 2011.
9. Green J, Collins C, Kyzar E.J., et al. (2012). Automated high-throughput neurophenotyping of zebrafish social behavior. *Journal of Neuroscience Methods*, 210(2), 266-271.
10. Kirkovski M, Suo C, Enticott P.G., et al. (2018). Short communication: Sex-linked differences in gamma-aminobutyric acid (GABA) are related to social function in autism spectrum disorder. *Psychiatry Research: Neuroimaging*, 274, 19-22.
11. Yin L, Jao L, Chen W. Generation of Targeted Mutation in Zebrafish Using the CRISPR/Cas System. *Methods in Molecular Biology*, 1332, 205-207.