22qll.2 deletion syndrome is the most common microdeletion syndrome that occurs in approximately 1 in 6000 live births (1,2). 22qll.2 deletion syndrome has many symptoms that range in severity. However, 90% of patients have learning and/or developmental disabilities (3), like autism (4). 22q11.2 is a hemizygous deletion that removes 30-40 genes on one copy of chromosome 22 (5). One of the genes located in this region is Transcript Box-1 (Tbx1). Tbx1 is a critical transcription factor that plays a role in cell differentiation (3) and Tbx1 protein levels correlate with severity of various phenotypes associated with the syndrome (1). Tbx1 levels are also sensitive to environmental changes, like the presence of the chemical compound 6-propyl-2-thiouracil, which decrease Tbx1 levels (6). In 22q11.2 deletion syndrome patients with autism, Tbx1 has been shown to regulate the formation of embryonic neurons (4), yet the role of Tbx1 in neurogenesis has not been fully determined.

I hypothesize that Tbx1 expression levels influence neurogenesis by interacting protein phosphatases during neuronal development. PTEN, a protein phosphatase, has been linked to autism characteristics when knocked down (7) and interacts with Tbx1 during neurogenesis. The primary goal of this research is to further understand the relationship between 22q11.2 deletion syndrome and autism.

Aim 1: Identify Tbx1- protein phosphatase interaction partners that regulate neuronal development in early embryogenesis
Approach: I will perform TAP-Tag mass spectrometry to identify novel Tbx1 binding partners in the brain of zebrafish. I will then knockout the identified phosphatases using CRISPR/Cas9 to determine if they play a role in neurogenesis. **Hypothesis:** Tbx1 will interact with other proteins phosphatase in brain tissue, and neurons and social behavior will appear abnormal when these factors are knocked down. **Rationale:** Tbx1 is known to interact with PTEN, a neuronal phosphatase that is associated with symptoms of autism (7).

Aim 2: Determine how dephosphorylation regulates Tbx1 activity
Approach: Using NetPhos and ClustalW, I will identify likely phosphorylation sites along the Tbox domain in zebrafish. I will then use CRISPR/Cas9 to individually substitute each site with an amino acid that always phosphorylated. **Hypothesis:** Tbx1 is being activated by protein phosphatases and the amino acid substitution will result in abnormal neuron formation and behavior. **Rationale:** Protein phosphatases can either activate or deactivate proteins via dephosphorylation (8).

Aim 3: Determine if knocking down Tbx1 regulates autism-like behaviors
Approach: I will expose varying concentrations of 6-propyl-2-thiouracil to early stage zebrafish embryos. Brain development and social behavior will be compared to the PTEN knockout. **Hypothesis:** As the concentration of 6-propyl-2-thiouracil increases, neurons will not form properly and zebrafish will experience antisocial behaviors similar to the PTEN knockout. **Rationale:** This will determine if low levels of active Tbx1 is sufficient to cause autism symptoms and 6-propyl-2-thiouracil decreases Tbx1 levels in the brain without other lethal side effects (6).

These experiments will provide information on if a phosphorylation pathway regulates Tbx1 and if low active Tbx1 level in the brain is sufficient in causing autism characteristics.

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