Idiopathic scoliosis affects 3% of adolescents worldwide, and is characterized by the spine being curved sideways in a C- or S-shape[1]. This curvature causes back pain, and the asymmetric ribcage often puts pressure on the lungs and heart[2]. Mutations in the protein-tyrosine kinase-7 (*ptk7*) gene are associated with idiopathic and congenital scoliosis. Ptk7 is a critical regulator of the Wnt/PCP (Wingless Integrated-Planar Cell Polarity) signal transduction pathway, which generates waves of traveling gene expression along the posterior body axis during early embryonic development[3]. *Due to a lack of research on model organisms with spine structure sufficiently similar to humans, the details of the interaction between ptk7 variants and the Wnt signaling pathway remain unclear*[3].

I **hypothesize** that ptk7 variants lead to scoliosis by altering the regulation of several proteins to collectively act as an on/off switch for the Wnt signal transduction pathway, altering planar cell polarity in cells involved in bone development. The **long-term goal** of this study is to further our understanding of the details regarding the underlying mechanism of scoliosis pathogenesis. The **objective** is to utilize the zebrafish model to uncover the role of ptk7 in the Wnt/PCP signaling pathway. The zebrafish is a good model organism for this study because the cranial-to-caudal pressure from swimming forward is comparable to the downward pressure gravity imposes on humans while exacerbating scoliosis[3].

Aim 1: Identify novel mutations in the ptk7 gene that cause idiopathic or congenital scoliosis. **Approach:** I will use next generation sequencing to locate several ptk7 variants in zebrafish with scoliosis. It will then be determined whether or not each specific variant actually contributes to the scoliosis phenotype after introducing single base mutations to embryos via CRISPR-Cas9. Each variant will have a group of zebrafish to be examined during the larval stage for signs of congenital scoliosis such as abnormal vertebrae size, shape, or alignment. Variant groups that do not display congenital scoliosis will then have their dorsal:ventral and left:right length ratios calculated during adolescence to detect for idiopathic scoliosis. Groups that begin displaying idiopathic scoliosis will be re-examined at several stages to measure the severity of disease progression for that variant. It will also be noted which variants never develop scoliosis. **Hypothesis:** I expect to find multiple sites of variation within the ptk7 gene, some of which will cause the scoliosis phenotype. **Rationale:** To better understand how ptk7 variants cause scoliosis, it is imperative to know which domains the mutations that cause each type of scoliosis are in, and it will be beneficial to determine which specific variants cause the most deleterious curvature.

Aim 2: Determine if mutant ptk7 is an inefficient regulator of skeletal development proteins. **Approach:** Gene ontology will be used to identify bone formation genes ptk7 regulates such as LRP6. Then RNA-seq will be used to compare the control group transcriptome to the transcriptomes of the variant groups. **Hypothesis:** Bone formation genes will be dysregulated in ptk7 mutants. **Rationale:** GO is a useful tool to identify groups of genes involved in bone development, and RNA-seq will allow us to quantify the difference in expression of those relevant genes when ptk7 is mutated.

Aim 3: Identify novel proteins that differentially bind to mutant ptk7. **Approach:** Co-immunoprecipitation will be used to perform a proteome-wide screen on a control group and variant groups. Proteins with the largest interaction differential will then be entered into the gene ontology database. **Hypothesis:** Mutant ptk7 will bind less ligands than wild type ptk7, and we will find novel proteins binding to ptk7 that are unrelated to the Wnt/PCP pathway. **Rationale:** Co-IP will allow us to measure the relative amounts of protein-protein interactions in the control and variant fish. Gene ontology can be used to determine the functions and processes of the proteins that are binding to ptk7 more or less often in the mutants relative to the normal fish.

With more knowledge of the specific ptk7 variants, a better idea of the bone development genes ptk7 regulates, and a thorough understanding of the alteration of protein interactions in mutant ptk7, we will be able to uncover the role of ptk7 in the Wnt/PCP pathway and begin research on therapies that could deter disease progression of scoliosis in millions of people worldwide.

1. Grimes DT, Boswell CW, Morante NF, Henkelman RM, Burdine RD, Ciruna B. Science. 2016 Jun 10;352(6291):1341-1344.
2. https://www.mayoclinic.org/diseases-conditions/scoliosis/symptoms-causes/syc-20350716
3. Hayes M, Gao X, Yu LX, Paria N, Henkelman RM, Wise CA, Ciruna B. Nat Commun. 2014 Sep 3;5:4777.