**Congenital heart disease (CHD)** accounts for the largest percentage of morbid congenital abnormalities in the world, affecting approximately 8/1000 newborns [1]. CHD is thought to be caused by the disruption of a complex network of cardiac regulatory genes and proteins which together dictate proper heart formation [2]. The NKX2-5 gene, a master regulator of heart formation in vertebrates, encodes a homeobox transcription factor that interacts with several heart developmental factors [3]. Previous studies have shown that specific mutations in NKX2-5 lead to characteristic defects of CHD, such as septal defects, arrhythmias or eventual heart failure [4]. *Despite this detailed knowledge of how specific mutations in NKX2-5 give rise to CHD defects, the role of NKX2-5 in cardiac morphogenesis and dysmorphogenesis remains poorly understood*.

My **primary goal** is to uncover the function of NKX2-5 in heart formation and CHD. The mouse model organism offers several advantages over other model systems [5], and will be used to study the function of NKX2-5. I **hypothesize** that NKX2-5 interacts with several transcription factors, and key mutations in both exonic and intronic regions disrupt these interactions, leading to CHD and heart malformities. My **long-term goal** is to determine the role of exonic and intronic mutations in the NKX2-5 gene necessary to develop characteristic defects of CHD. Such an insight would allow researchers to target these mutations through gene therapy, possibly offering cures or treatments to certain characteristic defects of CHD.

**Aim 1: Identify conserved mutations in the exonic and intronic regions of the NKX2-5 gene in orthologs with CHD.**

**Approach:** I will utilize MEME to identify amino acid sequences that are conserved in NKX2-5 between other model organisms and humans. Using CRISPR/Cas9, I will then induce mutations found in humans in the NKX2-5 gene in mice to determine if conserved intronic and exonic mutations of NKX2-5 in mice models lead to similar phenotypic defects in humans after examining the mice hearts after development.

**Rationale:** Conserved mutations in the exonic and intronic regions of NKX2-5 could reveal phenotypic differences across closely-related human orthologs. Depending on the conserved mutation along the NKX2-5 gene, this would imply that CHD is controlled from a regulatory mechanism (as in the case with conserved mutations in the intronic regions), post-transcriptional level, or that specific mutations along the NKX2-5 gene may dramatically alter the function of the NKX2-5 transcription factor, such as through a truncation or frameshift event.

**Hypothesis:** Specific conserved mutations along the intronic and exonic regions of the NKX2-5 gene will lead to more severe defects characteristic of CHD, such as septal defects or tetralogy of fallot.

**Aim 2: Determine how common NKX2-5 mutations, which give rise to CHD, influence interactions between NKX2-5 and other known cardiac regulatory genes/proteins**

**Approach:** CRISPR/Cas9 will be used to induce the most common known mutations to the NKX2-5 gene that give rise to characteristic defects of CHD in mice. Co-immunoprecipitation of all known cardiac regulatory proteins, such as TBX5 and GATA4, will be performed in the hearts of mutant mice with NKX2-5. A western blot analysis will then be performed to identify protein interactions with mutant NKX2-5 protein.

**Rationale:** Specific mutations in the NKX2-5 are known to give rise to specific defects characteristic of CHD. However, it is unclear how such mutations influence the role of NKX2-5 and its interaction with other cardiac regulatory genes to define proper heart formation. This approach will determine how common mutations known to occur in the NKX2-5 intronic and exonic regions alter the interaction of NKX2-5 with known cardiac regulatory proteins.

**Hypothesis:** Specific mutations in the NKX2-5 intronic and exonic regions will lead to decreased interaction of the NKX2-5 transcription factor with other well-characterized cardiac regulatory proteins, such as TBX5 and GATA4, leading to CHD.

**References**

1. What Are Congenital Heart Defects? (2011, July ). Retrieved from <https://www.nhlbi.nih.gov/health/health-topics/topics/chd>
2. Tanaka, M., Chen, Z., Bartunkova, S., Yamasaki, N., & Izumo, S. (1999). The cardiac homeobox gene Csx/Nkx2. 5 lies genetically upstream of multiple genes essential for heart development. *Development*, *126*(6), 1269-1280.
3. Schott, J. J., Benson, D. W., Basson, C. T., Pease, W., Silberbach, G. M., Moak, J. P., ... & Seidman, J. G. (1998). Congenital heart disease caused by mutations in the transcription factor NKX2-5. Science, 281(5373), 108-111.
4. Chung, I. M., & Rajakumar, G. (2016). Genetics of congenital heart defects: The NKX2-5 gene, a key player. Genes, 7(2), 6.
5. Lambrechts, D., & Carmeliet, P. (2004). Genetics in zebrafish, mice, and humans to dissect congenital heart disease: insights in the role of VEGF. *Current topics in developmental biology*, *62*, 189-224.