**Specific Aims**

There are many genes in the human body responsible for efficient running economy, which can be quantified by measuring energy utilization while moving at an [aerobic](http://en.wikipedia.org/wiki/Aerobic_exercise) intensity. The gene of interest to this project is COL5A1. COL5A1 codes for the protein pro-alpha-1(V) chain. Three of these proteins come together to form a molecule of type V collagen. Type V collagen helps to strengthen and support the extracellular matrix as well as many tissues such as ligaments, tendons, and muscles. Type V collagen also plays a role in forming these structures with type I collagen. COL5A1 is conserved in most organisms and is necessary for survival during development. DNA sequencing has shown that a single nucleotide polymorphism (SNP) in the 3' untranslated region (UTR) of COL5A1 is linked to enhanced running endurance. This variation has been labeled rs12722. It is unknown how the rs12722 gene product differs from the normal COL5A1 protein to produce an increase in inflexibility, a trait that promotes a more efficient running economy.

The **objective** of this study is to better understand how this variant protein contributes to an improved running economy by looking at its role in collagen fibril formation. There is speculation that rs12722 increases the production of type V collagen, allowing the protein to produce more collagen fibrils that could increase inflexibility of ligaments and muscles.

**The hypothesis for this study is that the rs12722 gene product enhances running economy due to an increase in fibril formation as a result of higher transcription and translation. This will promote extracellular matrix support and therefore create more muscle inflexibility.** It is known that type V collagen is the precursor to collagen fibril assembly in some muscles. The fibrils made from pro-alpha-1(V) provide the major biomechanical scaffold for cell attachment and allow for the shape and form of tissues to be maintained.

The **long term goal** of this study is to better understand the specific mechanism that causes rs12722 type V collagen to improve running economy. The best way to achieve this is to reach a better understanding of how the rs12722 pro-alpha-1(V) chain affects functionality of ligaments in the body.

**Specific Aims:**

**1. To determine if the SNP is within a DNA motif or if a miRNA exists that would bind to the area of the normal 3' UTR region of COL5A1 mRNA that contains the base pair where the SNP would be.** This can be tested by using the databank DREME, which contains known DNA motifs. miRBase can be used to determine if there is a miRNA that would bind with the rs12722 mRNA to regulate translation as well.

**Rationale:** If the SNP is within a motif region of the 3' UTR of the COL5A1 gene, then perhaps that somehow alters a region of DNA or mRNA to allow more pro-alpha-1(V) chain to be produced.

**2. To determine if there are higher levels of rs12722 mRNA compared to normal COL5A1 mRNA in smooth muscle cells.** By producing homozygous mutant mice, heterozygous mice, and wild type mice, RNA Sequencing of ligament tissues in mice can be used to see if more copies of the rs12722 mRNA are being transcribed compared to the normal mRNA.

**Rationale**: COL5A1 gene is highly conserved in mice compared to humans. By seeing if there is more rs12722 present, an assumption could be made that the quantity of type V collagen in ligament tissues somehow is related to the increase in inflexibility which produces an enhanced running economy.

**3. To determine if there are any new proteins that interact with rs12722 variant gene product.** By using a TAP tag on rs12722 variant, I can see if there are any noticeable or new protein interactions with the rs12722 protein that are not present in normal pro-alpha-1(V) chain. I can compare the results to the current known protein to protein interaction network of pro-alpha-1(V) chain.

**Rationale:** There could be a protein that interacts with the rs12722 protein, but not with the normal COL5A1 gene product, that promotes an increase in extracellular matrix support. This in turn could increase inflexibility.