**Specific Aims**

There are many genes in the human body responsible for efficient running economy, which can be measured by energy utilization while moving at an [aerobic](http://en.wikipedia.org/wiki/Aerobic_exercise) intensity. The one 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. This helps to strengthen and support many tissues such as ligaments, tendons, muscles, and the extracellular matrix and also plays a role in forming 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 COL5A1 mutation affects cellular functionality to produce this trait.

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 with type I collagen. There is speculation that rs12722 increases protein stability, allowing type V collagen to efficiently absorb energy produced by impact from running.

**The hypothesis is that type V collagen produced by rs12722 does enhance running economy due to a more durable collagen fibrils and a more stable interaction with type I collagen.** 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 allows 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. To achieve this, a better understanding of how this rs12722 pro-alpha-1(V) chain effects functionality of ligaments in the body. By performing the experiments described in the specific aims, some of the gaps in knowledge will hopefully be filled.

**Specific Aims:**

**1. Analyze the human DNA sequence of COL5A1 to see if the SNP is within a DNA motif or regulatory region that effects transcription.** This could determine whether the stability of the protein is due to a more stable RNA transcript or if the end product protein contains a stronger domain in which to bind type I collagen. This can be tested by using the databanks online containing known DNA motifs and seeing if the SNP is located within one.

**2. Analyze the transcriptome of mice embryos to see if more of rs12722 is being produced in ligaments.** By producing homozygous mutant mice, heterozygous mice, and wild type mice, RNA Sequencing of ligament tissues in mice embryos can be used to see if more copies of the rs12722 pro-alpha-1(V) chain are being produced compared to the normal pro-alpha-1(V) chain.

**3. Use Mass Spectroscopy to see if the ratio of type I collagen to type V collagen is higher when the type V collagen is produced by rs12722 .** By analyzing the ligaments of mice embryos, we can use mass spectroscopy to see if more type I collagen is recruited in collagen fibrils that started with rs12722 type V collagen.