**Specific Aims**

There are many genes in the human body responsible for an efficient running economy, which can be defined as running performance measured by energy utilization while moving at an [aerobic](http://en.wikipedia.org/wiki/Aerobic_exercise) intensity. The one of interest to this study is COL5A1. This gene codes for a component of type V collagen (called the pro-alpha1(V) chain) that is used 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. Three of these components come together to form a molecule of type V collagen. DNA sequencing has shown that a single nucleotide polymorphism (SNP) in the 3' untranslated region (UTR) of this gene has been linked to enhanced running endurance. This variation has been labeled rs12722 and has already been linked with inflexibility, a trait that actually benefits long distance runners. It is unclear whether the mutation affects a portion of the DNA that codes for regulatory regions that influence translation efficiency, localization, or stability of the mRNA. There is speculation that this polymorphism causes a more stable form of the protein to be made, therefore allowing for the collagen to endure for longer and be more energy efficient, but also reducing flexibility of ligaments. While there is research that supports this enhanced running economy phenotype, there has also been some conflicting studies to the validity that this gene does cause this phenotype.

**The hypothesis for this assignment is that the type V collagen produced by this variant of COL5A1 does in fact enhance running economy due to a more durable protein and a more stable interaction with type I collagen, which causes the inflexibility that has been noted in other studies.** This hypothesis was formed based upon the many different studies on the gene and it's connection to enhanced running by looking at marathon and triathlon runners. It is also known that type V collagen is the precursor component to collagen fibril assembly in some muscles. The fibrils provide the major biomechanical scaffold for cell attachment and allows for the shape and form of tissues to be maintained. A study between the two variations with an in depth look at the relationship between the two collagens has not been performed.

The **long term goal** for this assignment is to better understand the role type V collagen plays in muscle tissues and how the 3' UTR of the COL5A1 helps to code for a more stable protein. The **objective** of this study is to first confirm that this gene plays a role in improved aerobic running, and if that is the case then study specifically how this variant protein contributes to an improved running economy by looking at its role in collagen fibril formation with type I collagen. This will be achieved by completing the specific aims listed below.

**Specific Aims:**

**1. Confirm that this variant of COL5A1 increase long distance running capabilities.** As stated before, there are conflicting studies on the phenotype of this gene. To confirm this, a larger sample size will be required as the largest study to confirm this was approximately 400 marathon runners. Ideally there would be over 700 runners for this experiment. Blood will be drawn from participants, where their DNA will be sequenced using Next-Generation Sequencing and genotyped. Runners will be asked to perform the sit and reach range of motion (SR ROM) test to measure for flexibility as well. After the times for the runners have been recorded, we will compare the genotypes of the members of the groups, with times and scores for the SR ROM tests.

**2. Analyzing the human DNA sequence of COL5A1 to see if the SNP is within a DNA motif or regulatory region.** This could confirm 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 within the area.

**3. Perform and in vitro experiment to test stability of interaction between rs12722 type V collagen and type I collagen.** By performing an in vitro experiment, we can set up the conditions for fibril formation to take place. By doing so, we can analyze the protein-protein interaction that takes place between the variations of type V collagen and type I collagen to determine the strength of the structure.