



Mutations and Disease Mutations in the Myosin Gene

Goals

- Explore how mutations can lead to disease using the myosin gene as a model system.
- Explore how changes in the amino acid sequence of a protein could affect its function.
- Understand that DNA is the coded material that tells us how to make all our proteins, whereas proteins are the molecules that actually carry out all our functions.
- Understand that DNA has a very uniform structure, regardless of its sequence, whereas proteins vary widely in structure, allowing them to perform a wide variety of very different functions.
- Read a DNA sequence and translate it into an amino acid sequence.

Background –

Familial hypertrophic cardiomyopathy is an inherited heart disorder leading to thickening of the left ventricular wall, decreased heart function and shortness of breath. Additionally, affected individuals often die suddenly of cardiac failure.

It is known that one of the causes of hypertrophic cardiomyopathy is a defect in one of the proteins responsible for heart muscle contraction; cardiac myosin. In order to better understand the molecular defects leading to familial hypertrophic cardiomyopathy, two different families were followed. Family A resides in the United Kingdom. One member of this family suffered heart failure at age 21 and was resuscitated. Family B is of Japanese ancestry. A member of this family died of cardiac complications at age 40. In the exercise that follows you will examine differences between affected and unaffected individuals from each of these families.

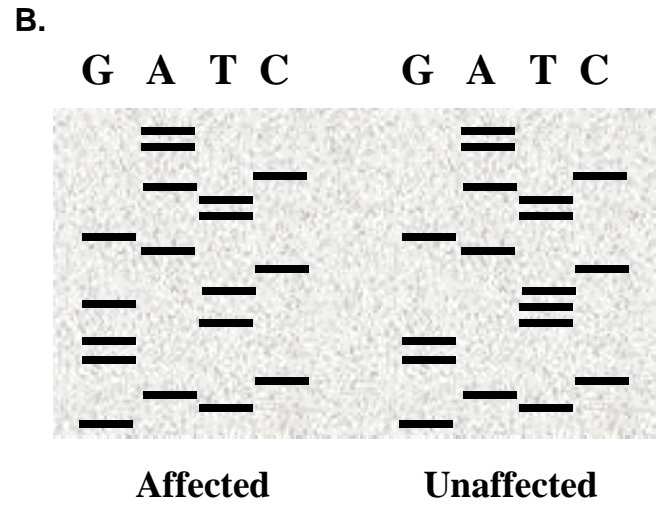
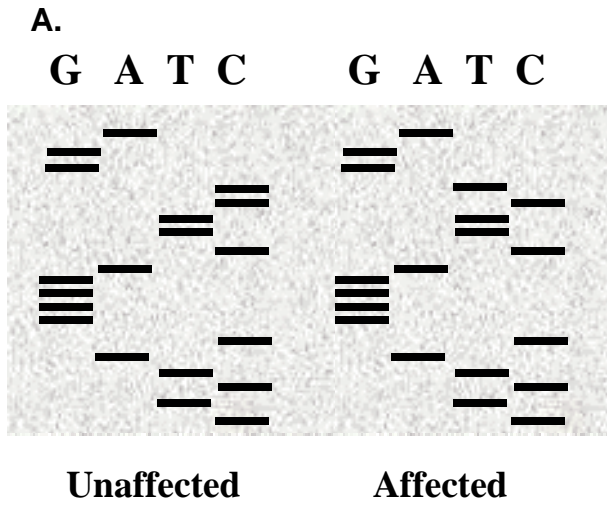
Step 1 – Finding the mutation

The cardiac myosin gene was sequenced from both affected and unaffected members from the two different families. Below are pieces (the actual sequence is much longer, over 6,000 base pairs) of the sequencing gels. Note that different parts of the same gene are shown for the two different families.

Gel A shows bases 2140 – 2157 representing amino acids 714 – 719.

Gel B shows bases 1531 – 1548 representing amino acids 511 – 516.

Read the nucleotide sequences from the gels and note them on the following page (remember the sequence should be read from bottom to top). Circle the nucleotide that differs between the unaffected and affected individuals.



Family A. Unaffected

Family A. Affected

Family B. Unaffected

Family B. Affected

You have found two different mutations in the cardiac myosin gene. One of these mutations is present in Family A, the other is present in Family B. Next you will examine whether the change in nucleotide sequence leads to a change in amino acid sequence of the beta myosin protein.

Step 2 – Translate the DNA sequence into an amino acid sequence

Using the genetic code provided, translate each DNA sequence into an amino acid sequence (note your answers below the DNA sequence you recorded on the previous page).

- Assume that the first codon begins with the first base you can read.
- Assume that you are reading the coding strand (there is no need to generate the complement).
- Remember to substitute Us for Ts since the genetic code provided is for RNA rather than DNA.

Circle the amino acid that differs between the unaffected and affected alleles.

Question 1

Do both of these mutations lead to a change in amino acid sequence?

Naming Mutations

There is a specific way in which scientists name mutations.

- The amino acids in a protein are numbered sequentially from the amino terminus to the carboxyl terminus. Myosin has 2002 amino acids that are numbered from 1 through 2002.
- The number in the mutation name describes the position of the amino acid that is altered in the mutant protein.
- The letters in the mutation name refer to amino acids. Scientists use a one-letter code to represent each of the 20 amino acids. That code is shown below.
- The letter before the number in the mutation name refers to the amino acid that would be at this position in the normal myosin protein.
- The letter after the number refers to the amino acid that has been put in place of the normal amino acid in the mutant myosin protein.

Example

P574H - The proline (P) usually found at position 574 is replaced by a histidine (H).

Single letter amino acid code

G	Glycine	P	Proline
A	Alanine	V	Valine
L	Leucine	I	Isoleucine
M	Methionine	C	Cysteine
F	Phenylalanine	Y	Tyrosine
W	Tryptophan	H	Histidine
K	Lysine	R	Arginine
Q	Glutamine	N	Asparagine
E	Glutamic Acid	D	Aspartic Acid
S	Serine	T	Threonine
X	Stop		

The sequence you are dealing with for Family A covers amino acids 714 – 719.
The sequence you are dealing with for Family B covers amino acids 511 – 516.

Questions 2 - 9

In Family A, what is the position number of the amino acid that is changed?

In Family A, what amino acid is usually found at this position?

In the affected member of Family A, what amino acid is found at this position?

How would a scientist name this mutation?

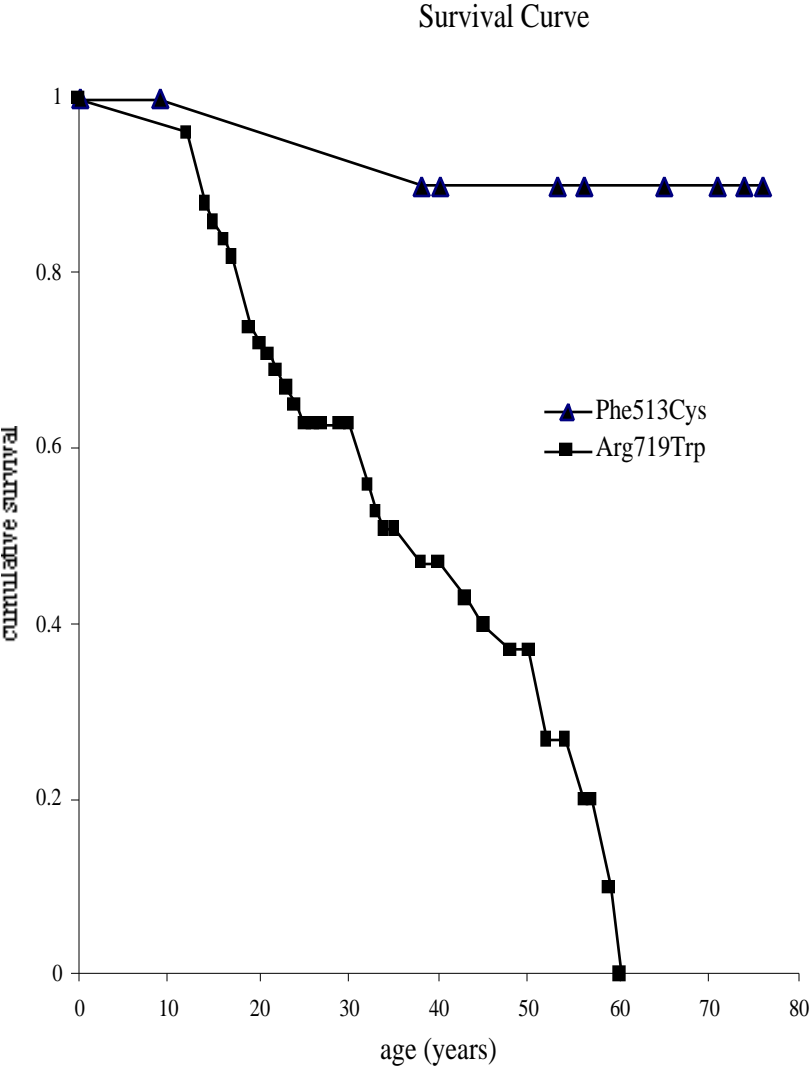
In Family B, what is the position number of the amino acid that is changed?

In Family B, what amino acid is usually found at this position?

In the affected member of Family B, what amino acid is found at this position?

How would a scientist name this mutation?

Step 3 - Next, we will look at how the two different mutations affect the heart and survival of individuals with these mutations. Examine the survival curve for the two different mutations you have been working with.



A curve showing the survival of individuals who have the R719W or F513C mutation. Each spot represents a person. The age at which the spot appears shows the present age of a living person or the age at which the person died. The R719W data is a combination of data from four unrelated families with the same mutation.

Question 10

What difference do you observe between the two curves? Which mutation has a bigger affect on survival?

Next you will try to use the data you have to explain the difference in survival.

Step 4 – Proteins are composed of long chains of amino acids.

Unlike DNA, whose 3-dimensional structure is quite uniform, proteins fold in many unique shapes, leading to the specificity that allows proteins to interact with one another and other molecules in precise manners. Each protein (say myosin) will assume only one very specific 3-dimensional structure that is different from that of all other proteins (actin, hemoglobin, insulin etc).

The 3-dimensional structure of a protein is determined by the order of amino acids in the protein. Different amino acids have different side chains with different size, shape, polarity and charge. A protein will fold such that negatively charged amino acids are near positively charged amino acids. Also, the protein will fold such that hydrophobic amino acids are near other hydrophobic amino acids, and hydrophilic amino acids are near other hydrophilic amino acids.

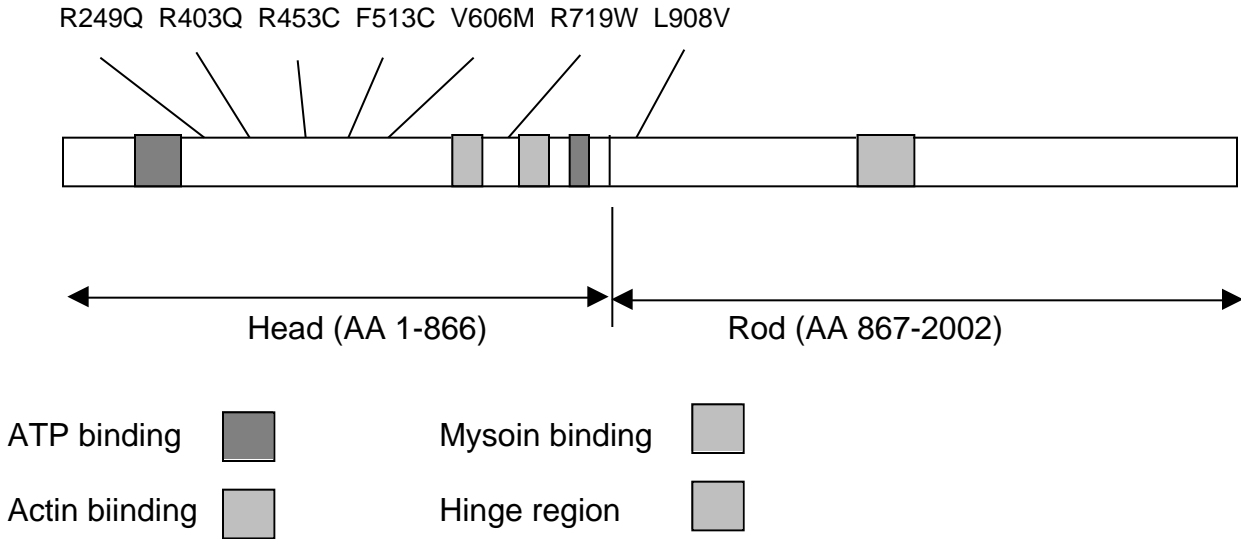
If the amino acid sequence of a protein is changed, the 3-dimensional structure of the protein may change as well. If an amino acid is replaced by an amino acid of different charge, polarity, size or shape, the protein may not be able to fold in the same exact manner. This can result in a complete or partial loss of function of the protein.

Question 11

Look at the structure of the amino acids involved in the changes in the different families with hypertrophic cardiomyopathy (see accompanying handout for structure of the amino acids). Do any of the changes involve a change in size, shape, polarity or charge?

Mutations in a region critical for the function of a protein are more likely to have a detectable affect on the activity of the protein than mutations in non-essential areas.

Below is a map of the myosin protein. The different parts of the protein that play important functional roles have been determined and are shown on the map as boxes of different color.



The portions of the protein representing the head and rod regions are shown below the map. Remember that myosin fibers are formed of many rods together with the heads pointing out towards the actin filaments. It is the myosin heads which interact with actin.

The positions of many different known mutations in myosin are shown above and below the map.

Question 12

Look at the position of the amino acids involved in the changes in the different families with hypertrophic cardiomyopathy. Are any of the mutations in an area critical for the function of myosin?

Question 13

Using all the information you have so far – list at least two possible explanations for the difference between survival curves with the different mutations.

1.

2.

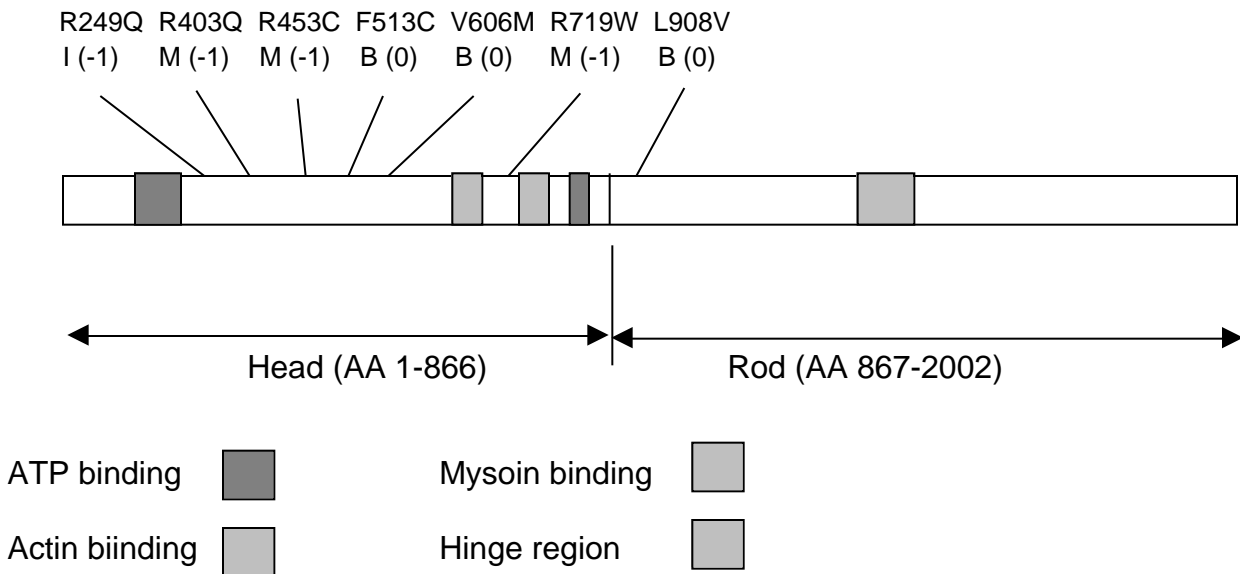
Summary of known work on myosin mutations

The work described in this activity is in progress and no one knows “for sure” why the Arg719Trp mutation has a much more profound affect on survival than the Phe513Cys mutation. However, a good deal of work has been performed in the field and some educated guesses can be made.

Scientists have divided all the mutations known to date in familial hypertrophic cardiomyopathy (FHC) into three groups;

- benign (B) or those associated with normal life expectancy
- malignant (M) or those which significantly decrease life expectancy
- intermediate (I) or those with a moderate effect on life expectancy.

Shown below is a map of the myosin gene, identical to the last map except that this time the classification of each mutation (B, M, or I) is shown, as well as the change in charge at that position which is caused by each mutation (shown in parentheses).



Two factors appear to be important in determining whether a mutation will have a noticeable negative effect –

- whether the mutation results in a change in charge of the amino acid
- whether the mutation occurs in a region of the gene essential for the function of the protein, (ATP binding or actin binding)

Mutation Phe513Cys, a benign mutation, does not result in a change in the charge of the amino acid. It also does not occur in a region of myosin critical for its function.

In contrast, mutation Arg719Trp, the malignant mutation we examined, results in a change in the charge of the amino acid (arginine has a charge of +1, tryptophan has no charge, resulting in a net change in charge of -1). This mutation is also located immediately next to the actin binding site.

Genotype and Treatment Options

Although there is currently no cure for familial hypertrophic cardiomyopathy we occasionally use genotype information in deciding how to treat people with this disorder. People who have serious symptoms and active disease are usually treated for symptom relief regardless of genotype. However, in some cases, individuals remain asymptomatic until sudden heart failure during exercise at a young age. In these cases, individuals carrying more serious mutations are often treated preventively. **Because we can test people for specific mutations, we can decide whether to treat them prior to the onset of symptoms in order to prevent serious consequences!!**

Muscle Contraction with Mutated Myosin

Question 14

This form of familial hypertrophic cardiomyopathy is inherited in an autosomal dominant fashion. What percentage of myosin molecules do you expect to be “mutant” in people with this form of familial hypertrophic cardiomyopathy?

Repeat the muscle contraction exercise.

This time 50% of the actin binding sites on myosin will be slightly different representing the Arg719Trp mutation, close to the actin binding site and leading to a change in charge at that position.

Questions 15 - 18

How did the Arg719Trp mutation affect muscle contraction?

Why might the heart be larger in people with the Arg719Trp mutation (familial hypertrophic cardiomyopathy)?

How would muscle contraction have been affected if all the myosin molecules had the Arg719Trp mutation?

Considering what you know about muscle contraction, could mutations in sarcomere proteins other than myosin result in problems similar to those seen with the Arg719Trp mutation? In what proteins might these mutations occur?