NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Dystrophin Stability and Cardiomyopathy

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Part I – Diagnosis

Jackson Young, a six-year-old kindergarten student, has been having trouble breathing while playing sports. At recess his teacher notices something is wrong because when he comes back in from playing he is very fatigued and has difficulty catching his breath. His teacher, Mrs. Blake, decides to call his parents. Later that night Mr. and Mrs. Young sit down with Jackson to discuss the situation.

Mr. Young: Hey Jackson, Mrs. Blake called and told us you were having trouble breathing after recess. Can you tell us what happened?

Jackson: I... I was just really tired I think.

Mrs. Young: Now Jackson honey, can you tell us more regarding what's going on?

Jackson: Well, when I was playing during recess, my chest started to hurt, and I couldn't catch my breath.

Mr. Young: Okay Jackson, if this happens again let us know.

Next week, after struggling again at recess, Jackson let his parents know and his parents took him to his pediatrician. The doctor prescribed an inhaler because she thought that the breathing trouble was due to asthma.

The following week at recess, Jackson decided he wanted to try and play basketball with his pals. But, after running for only a short period of time, Jackson started to feel extremely tired and out of breath. So, doing as the doctor had said, he took a puff out of his inhaler. But this didn't help Jackson at all. Jackson then told his teacher, Mrs. Blake, who sent Jackson to the school nurse.

Nurse: Hey there Jackson, what seems to be the problem?

Jackson: *(Trying to catch his breath.)* I..., I couldn't breathe, and I was dizzy, so I took a puff of my inhaler like the doctor told me and it hasn't gotten better.

Nurse: Okay, sit down while I call your parents.

[Ring… Ring...]

Mrs. Young: Hello?

Nurse: Hello Mrs. Young. I'm here with Jackson who is experiencing some chest pain, trouble breathing, and dizziness. He says he used his inhaler and his conditions haven't gotten any better.

Mrs. Young: Oh my, thank you for letting me know, I'm coming to pick him up now.

After the phone call, Mrs. Young told Mr. Young and called the pediatrician. On hearing this, the pediatrician recommended they bring Jackson to a specialist, Dr. Lock, and she set up an appointment.

Dr. Lock: Hello Mr. and Mrs. Young, what brings you in today?

Mrs. Young: Well, Jackson has started to exhibit symptoms such as trouble breathing, chest tightness and now dizziness when playing. We took him to his pediatrician, and she thought it was asthma and prescribed an inhaler, however, when Jackson used his inhaler at recess his symptoms didn't improve.

Dr. Lock: Let's take a look. Hmmm... Well, I notice some swelling in Jackson's lower legs and feet. I'm going to begin his blood work and run a few initial tests on Jackson including an EKG and chest X-ray.

After looking over the test results, the initial test indicated that Jackson had cardiomyopathy. Further genetic testing confirmed the diagnosis for X-linked dilated cardiomyopathy, a type of muscular dystrophy. Muscular dystrophies are a group of muscle diseases caused by mutations in a person's genes.

Dr. Lock: The chest x-ray shows that Jackson has an enlarged heart. Here, let me explain. Take a look at this diagram of a normal heart compared to an enlarged heart. *[Figure 1]* Now look at Jackson's x-ray. See how the left ventricle is enlarged? *[Figure 2]*

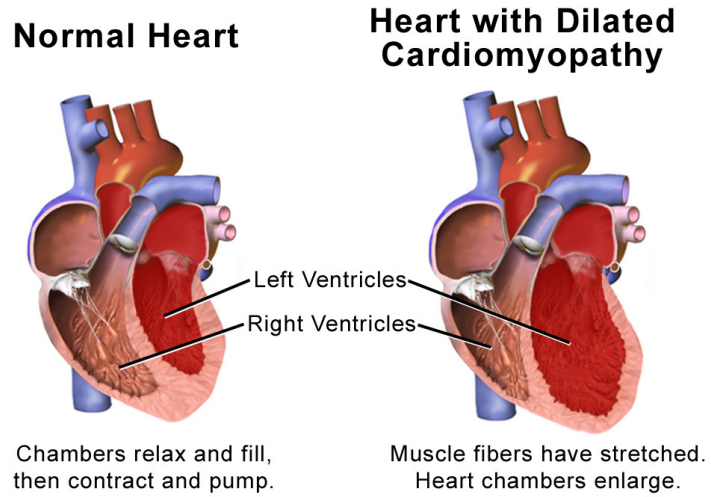


Figure 1. Diagram comparing normal heart and heart with dilated cardiomyopathy. *Credit:* Blausen.com staff (2014), Medical Gallery of Blausen Medical 2014, *WikiJournal of Medicine* 1(2), CC BY 3.0, <https://doi.org/10.15347/wjm/2014.010>.

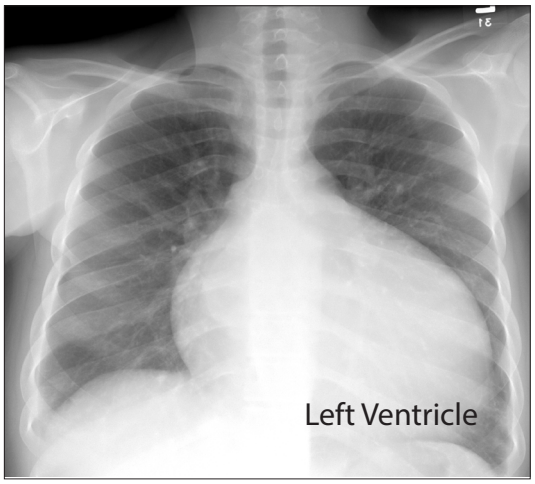


Figure 2. Chest X-ray showing enlarged heart. *Credit:* Courtesy of Hani Makky Al Salam, Radiopaedia.org, rID: 10761, modified CC BY-NC-SA 3.0, <https://doi.org/10.53347/rID-10761>

Dr. Lock: The genetic test results confirmed Jackson has a genetic variation in the gene that codes for the protein dystrophin. Dystrophin plays an important role in skeletal and cardiac muscle; this protein is part of a group of proteins (a protein complex) that work together to strengthen muscle fibers and protect them from injury as muscles contract and relax.

Mrs. Young: Oh my goodness, how can this be possible?

Dr. Lock: A variation in this gene can cause cardiomyopathy, which means an enlarged and weakened heart. Let's look at some information on dystrophin and its role in the body. This pamphlet *[see next page]* includes a drawing *[Figure 3]* that shows the protein dystrophin in purple. The rest of the pamphlet discusses that there are many possible mutations of the gene that can cause a range of muscle dystrophy disorders. Our next step would be to know the exact change in the protein dystrophin to understand Jackson's symptoms.

Dystrophin and Muscular Dystrophy

Muscular dystrophy is a group of degenerative diseases that are caused by mutations in a gene that lead to progressive weakness and muscle loss. These disorders differ by what muscles are affected, how fast they progress, and time of onset. Mutations in the gene encoding dystrophin on the X-chromosome lead to a defective link between the cytoskeleton and extra-cellular matrix of muscle leading to muscles that are more susceptible to damage.

Muscular dystrophy occurs in about one in every 3,600 males born worldwide with a prevalence of 63 cases per million. Depending on mutation the symptoms of muscular dystrophy can include muscle pain and stiffness, heart problems (cardiomyopathy, arrhythmias), trouble breathing, delayed growth, trouble walking, scoliosis, respiratory issues (increased risk of pneumonia, weak chest-wall muscles), and shortened life span.

Dystrophin is a very large protein that binds to actin in the muscle cells and connects to a transmembrane complex that interacts with the extracellular matrix (ECM). This provides a mechanical link between the cellular cytoskeleton and the extracellular matrix of muscle (see Figure 3). This interaction between dystrophin, actin, and the complex linked to the extracellular matrix provides the muscle cell with much of its structural strength that allows the muscle cells to contract and move. Thus, dystrophin is essential to the structural stability of the myofibers.

Dystrophin has multiple protein domains. Protein domains are functional three dimensional structures that give a protein different abilities and capacities. Dystrophin operates by interacting with a variety of proteins. The actin binding domain (ABD; green in Figure 3) binds to F-actin playing a central role in contraction of muscle. Central rod domains have 24 spectrum-like repeat and 4 hinge domain repeats (in purple and yellow respectively) and bind a variety of proteins including membrane lipids, another F-actin binding site, and the microtubules. The Cys-rich domain and C-terminal domain (CTD; in teal) bind the transmembrane dystrophin associated protein complex that connects with extracellular matrix.

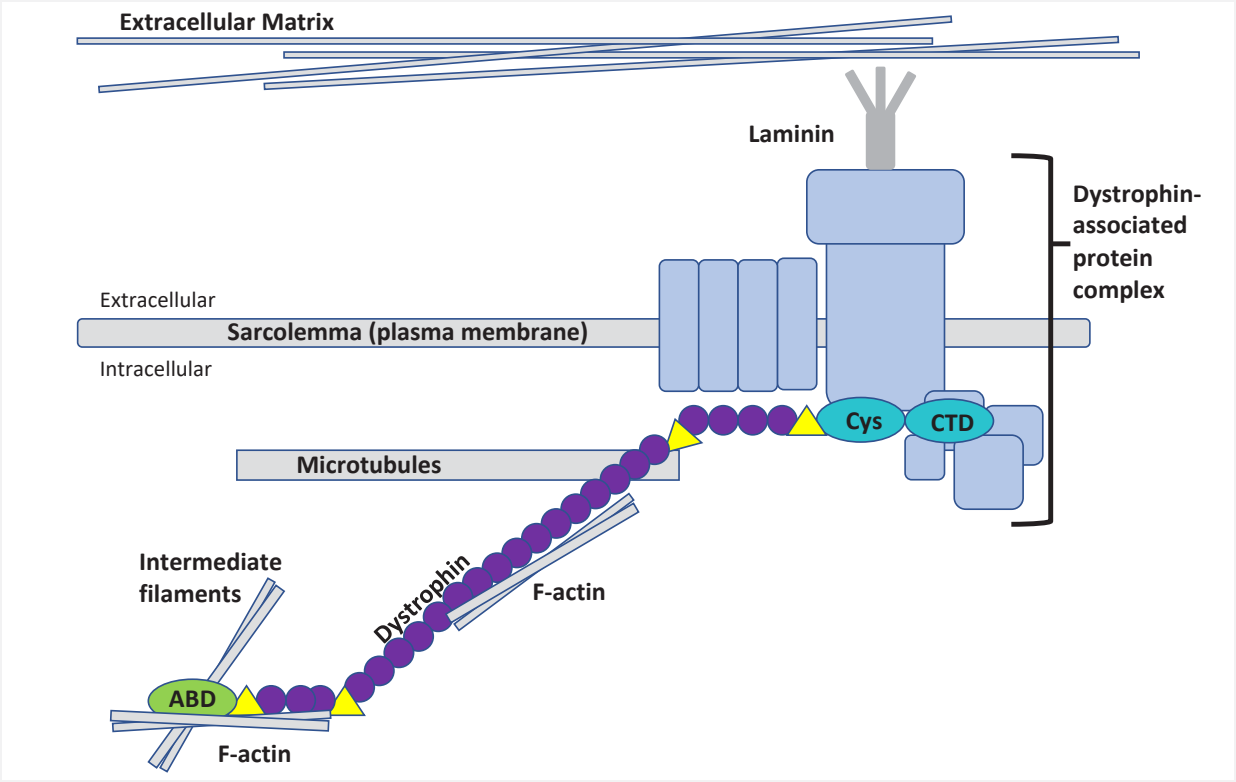


Figure 3. Dystrophin domain structure and protein-protein interactions. *Credit:* Adapted from Gao & McNally (2015).

Dr. Lock: Dystrophin helps in protecting and strengthening the muscle fibers as they contract. The loss of this protein can lead to things such as muscle wasting, difficulty breathing, and enlargement of the heart. While the right ventricle pumps blood to the lungs to pick up oxygen, the left ventricle has to pump the oxygenated blood to all the tissues of the body. When muscles contract, dystrophin works as a shock absorber. Mutations to the dystrophin gene can cause muscles to become weakened and damaged.

Mr. Young: How does changing one protein cause his heart to be weakened?

Dr. Lock: To answer that we can look at current research to better understand how this variation causes these symptoms.

Questions

1. What are the symptoms Jackson is experiencing?

2. How does cardiomyopathy affect the heart?

3. What is the role of dystrophin in the muscle?

4. What is the significance of protein domains?

5. What is the function of the actin binding domain in the protein?

Part II – Jackson's Specific Allele Variant

Based on the sequencing of the genetic test, Dr. Lock found that there is a variation in the sequence of the N-terminal actin binding domain of dystrophin. The genetic test showed a missense mutation that changes the 18th amino acid from a lysine (K) to an asparagine (N). There are many different variations or alleles of dystrophin that cause muscular dystrophy. Dr. Lock referenced a paper (Singh et al., 2014) that described the variant seen in Jackson, K18N, and compared it to the typical or "wild-type" N-terminal actin binding domain. The K18N variant effects cardiac muscle and does not affect skeletal muscle.

Dr. Lock: Jackson's cardiomyopathy is due to a variation in the sequence in the N-terminal actin binding domain. Here is a picture *[Figure 4]* that shows the ribbon diagram of the X-ray crystal structure of the wildtype and variant actin binding domain.

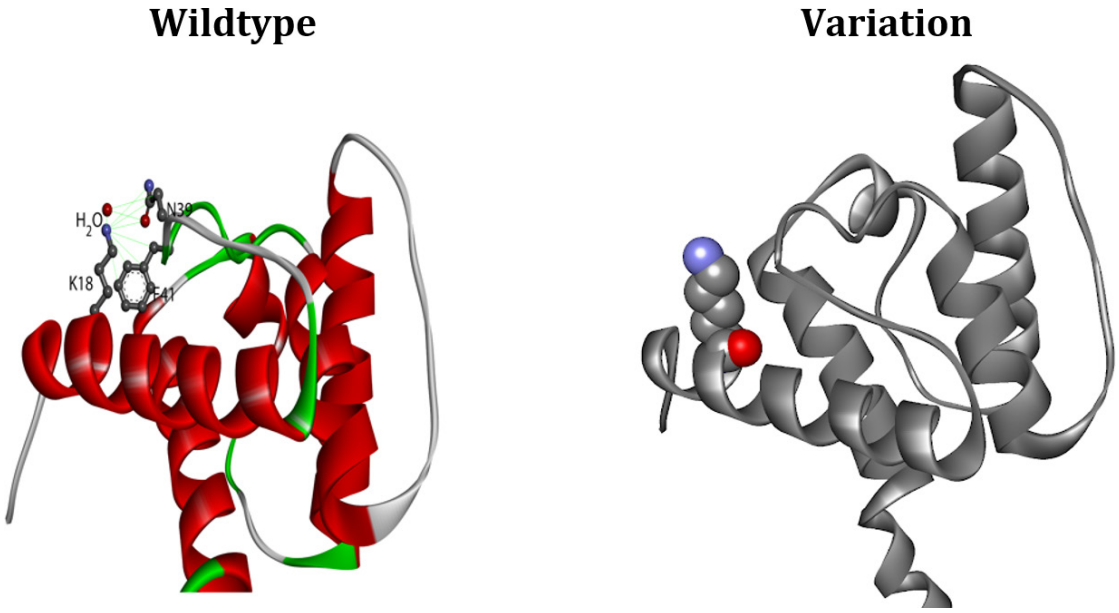


Figure 4. Dystrophin and variant structure. *Credit:* Adapted from Singh et al. (2014), CC BY 4.0.

Mr. Young: They look very similar to me.

Dr. Lock: Yes, good observation. There seems to be little difference in the structure between the two domains, and based on a gel electrophoresis test it was determined that there is no difference in the actin binding ability between the two types.

Mr. Young: If there is no difference in the function of the two proteins, then how is it affecting Jackson's health?

Dr. Lock: Proteins fold into a specific three dimensional conformation to form a functional protein. That structure can be disrupted by temperature, pH, and agitation.

Dr. Lock again referenced the paper by Singh et al. (2014) that conducted both thermal and denaturant melts on the wildtype and variant proteins to determine if there is a difference in the stability and unfolding of the proteins. Both the mutant and the wildtype purified proteins were put in a buffer system at the same concentration to understand stability. Thermodynamic and chemical stability were measured by circular dichroism spectophoscopy (CD). CD evaluates structure, folding, and binding properties of proteins. This type of spectroscopy can be read where a low absorbance indicates the folded native state of the protein. Increasing absorbance indicates a protein that has been unfolded. Comparing the wildtype and mutant makes it clear how the amino acid change effects stability. Thermal melts evaluate how changes in temperature effect protein stability and denaturant melts evaluate the effect of pH changes. The results are below (Figures 5 and 6).

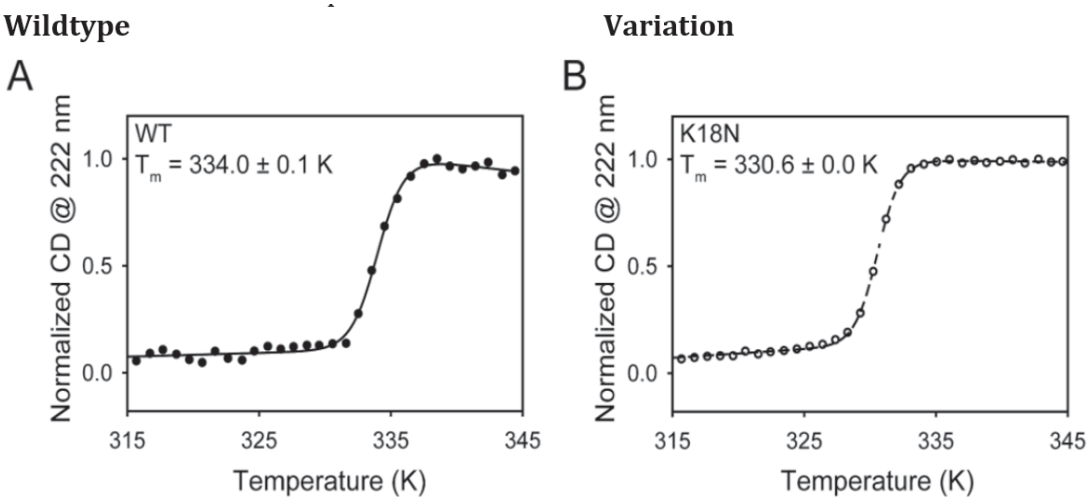


Figure 5. Thermal melts. T_m is the midpoint of thermal denature or unfolding. Converting the T_m to Fahrenheit shows that wild type T_m = 141 °F and the variant (K18N) is 135 °F. *Credit:* S.M. Singh et al. (2014), CC BY 4.0.

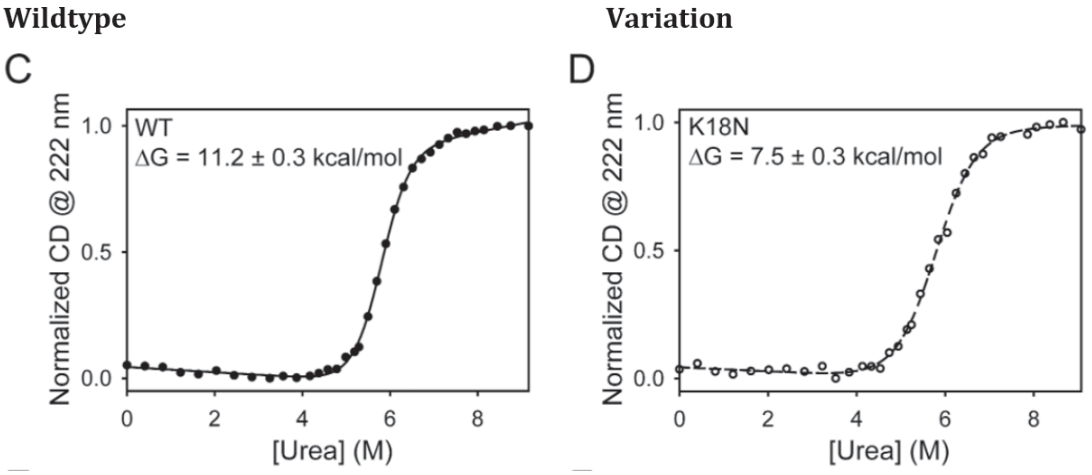


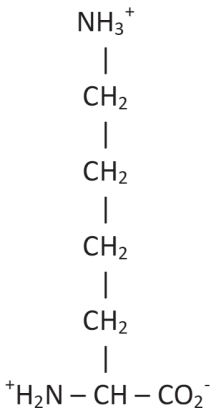
Figure 6. Denaturant melts. Note that ΔG is the free energy of unfolding. *Credit:* S.M. Singh et al. (2014), CC BY 4.0.

Questions

1. Examine the following amino acids (Figure 7, right) and then answer the questions below.
   a. Are the *R* groups of lysine and asparagine polar or nonpolar?

   b. Are the *R* groups of lysine or asparagine neutral, basic, or acidic?

Lysine Structure:



Asparagine Structure:

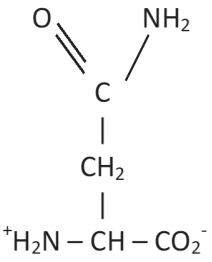


Figure 7. Lysine and asparagine structures.

c. What is the strongest bond the *R* group of lysine can participate in?

d. What is the strongest bond the *R* group of asparagine can participate in?

e. Using your answers from *c* and *d* above, determine which of those can form the strongest bond. Why?

2. How might the changes in the thermodynamic stability and pH stability change be explained by the mutation of a lysine to an asparagine?
   a. Does the wild type or the mutant have higher stability when comparing the thermal melts using CD? (This was calculated by modeling the temperature midpoint of the thermodenaturation transition known as the T_m.)

   b. Does the wild type or the mutant have higher stability when comparing the chemical denaturation using CD? (This was measured by the free energy of unfolding known as ΔG.)

   c. How might these changes in thermodynamic stability and pH stability be explained by the mutation of lysine to asparagine?

3. How can these changes affect the function of dystrophin and lead to Jackson's symptoms?

Conclusion

Dr. Lock: From these test results we can see that there is a difference in both the thermodynamic stability and the pH stability of dystrophin.

Mrs. Young: What does that mean?

Dr. Lock: It means that the mutated protein will fall apart at lower temperature than the normal one, causing it to lose its function. It also means that the mutated protein will fall apart quicker when introduced to a change in pH environment.

Mr. Young: Why does its stability in a different pH environment matter? Does the pH of our body change?

Dr. Lock: Well the normal pH of cardiac tissue is around 7. When we engage in physical activity, our body goes through lactic acid fermentation in order to produce energy. A biproduct of this process is lactic acid. Lactic acid lowers the pH of the surrounding tissues. This can be the reason why Jackson experiences these symptoms while participating in physical activity.

Mrs. Young: So what can we do to help Jackson?

Dr. Lock: Unfortunately there is no cure for cardiomyopathy. However, medicines can be prescribed to mitigate the symptoms Jackson experiences.

Mr. Young: Is this something he will be able to live with for a while?

Dr. Lock: Sadly, cardiomyopathy develops fast, so Jackson will most likely experience heart failure by the time he is 20. However, based on Jackson's overall good health and age there is the possibility for a heart transplant. This could allow him to live longer and medications could be prescribed to reduce the other symptoms associated with muscular dystrophy.

References

Blausen.com staff. (2014). Medical gallery of Blausen Medical 2014. *WikiJournal of Medicine* 1(2). <https://www.doi.org/10.15347/wjm/2014.010>

Gao, Q.Q., & E.M. McNally. (2015). The dystrophin domplex: structure, function, and implications for therapy. *Comprehensive Physiology* 5(3): 1223–39. <https://doi.org/10.1002/cphy.c140048>

Salam, H. (2010). Dilated cardiomyopathy. [Case study.] Radiopaedia.org. <https://doi.org/10.53347/rID-10761>

Sam, K., J. Feger, et al. (n.d.). Dilated cardiomyopathy. [Reference article.] Radiopaedia.org. <https://radiopaedia.org/articles/dilated-cardiomyopathy>

Singh, S.M., S. Bandi, D.D. Shah, G. Armstrong, & K.M.G. Mallela. (2014). Missense mutation Lys18Asn in dystrophin that triggers X-linked dilated cardiomyopathy decreases protein stability, increases protein unfolding, and perturbs protein structure, but does not affect protein function. *PLOS ONE* 9(10): e110439. <https://doi.org/10.1371/journal.pone.0110439>

Yue, P., Z. Li, & J. Moult. (2005). Loss of protein structure stability as a major causative factor in monogenic disease. *Journal of Molecular Biology* 353(2): 459–73. <https://doi.org/10.1016/j.jmb.2005.08.020>