



# The Pompe Predicament

How a Community of Scientists and  
Patients are Fighting for a Cure

UF Center For Precollegiate Education & Training

**UF** UNIVERSITY of  
**FLORIDA**

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# Author's Note

Pompe disease first entered my life in 2000. This seems like an eternity ago. I'm fortunate. I was simply a technician working in a research lab. I wasn't affected personally, and being in a research setting, I was insulated from the clinical implications of the disease. My PI was a molecular geneticist, in the days before the human genome was sequenced and you could ship a sample of your cheek cells off to a company for an analysis of your DNA. A fantastic part of science research is the collaboration among researchers and in a major research institute with a medical school that translates to academic medicine. Many clinicians are also researchers themselves or collaborate with research labs. In my instance, the clinician was a researcher, although his research focus is more on the animal model and physiologic responses of muscular disease. My lab was focused on molecular work.

I don't remember the details of the case. My best recollection is the clinician lost a pediatric patient in infancy, due to cardiac failure. The patient was diagnosed with Pompe disease. She had a twin who wasn't exhibiting symptoms of the disease yet. Were the siblings identical? Did they both possess mutations for Pompe disease? This was prior to whole genome sequencing. Certainly, you didn't just send your samples out as you do now. So, pretty much on the side, I proceeded to search GenBank for known mutations, develop PCR primer sets, and set about amplifying and searching the infant's DNA for the mutations.

I don't know how it ended up. Funding from another grant that provided my position ran out, and I moved on to another position. In my mind, they were fraternal twins and the surviving twin is a happy, healthy 11 year old.

As fate would have it, Pompe was in my view again, a decade later, although from a different perspective. Through a grant from the National Institutes of Health Science Education Partnership Award, my Center launched a professional development opportunity for high school educators, focused on translational research. Going from the bench to bedside and back again is the theme of this program, highlighting basic science and clinical advances. Pompe fits perfectly here as the University of Florida has a very strong gene therapy program and work with clinical trials involving enzyme replacement therapy for Pompe.

As I have researched to learn more about Pompe, two things have struck me: 1) the huge advances made in the diagnosis and treatment of Pompe patients and 2) the incredible world-wide Pompe community made up of patients, parents, researchers, clinicians, and even biotechnology companies. Pompe is a devastating disease, particularly for the youngest affected. With this curriculum, I hope teachers and students will gain an appreciation of the strength of the human enterprise as we forge ahead with advanced treatments and therapies for not just Pompe disease, but other human illnesses.

# Introduction

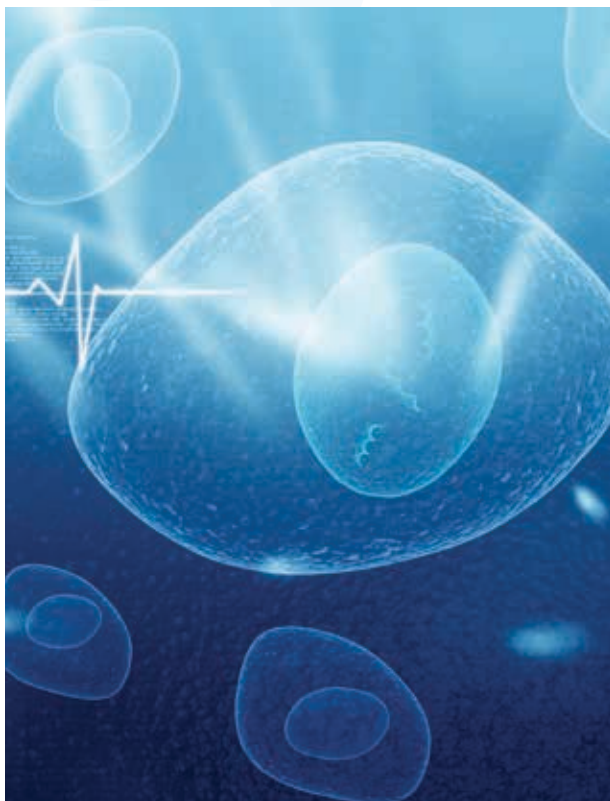
Pompe disease affords the rare opportunity for students to consider multiple biological concepts and assemble them into a story. Instead of DNA taught separate from protein structure and function, these areas as well as enzymes, genetics, and human disease are all taught together through the story of Pompe disease. Students can clearly see how mutations at the DNA level lead to an incorrect amino acid chain which then folds differently than the normal wild-type protein. These different structural confirmations result in an enzyme with reduced function. It is easier to understand that a protein with a different shape does not fit correctly when the students are visualizing 3D models. And those different structures are directly evident by the severity of Pompe disease. If you are currently using sickle cell disease or cystic fibrosis in your curriculum, you may want to consider adding Pompe to your toolbox.

My hope is that students will move through the unit and have their curiosity piqued. They will have the chance to think like research and clinical scientists and learn the basics of the disease and stop to consider how it might be treated. After brainstorming, students look at the historical record of Pompe research leading up to the current treatment and the next generation therapies in development. They will look at patient histories, perform diagnostic testing, and analyze the results of genome sequencing. After translating into amino acids, the students will use hands-on manipulatives to shape their specific protein, considering how amino acids attract or repel each other. They will hold their model and a normal acid alpha-glucosidase molecule and predict the functionality of the protein. As an extension, using the power of bioinformatics, the students can investigate further by querying the entire sequence, translate it into a string and three dimensional structure, rotate it on the computer and superimpose it with the normal molecule.

Also within this unit is the chance to explore ethical questions. Do we push the envelope a bit more and try developing therapies sooner when there is no other hope for a treatment or cure? Is it right to do this? To what extent are decisions based on good business practice? Pompe disease has a very strong patient advocacy group which has been involved in the development of enzyme replacement therapy, sometimes causing rifts between different groups, and questioning motives of stakeholders. Does this help or hinder development of the next generation of treatment? In the final activity, students consider the use of gene therapy and its promise as the only cure for Pompe disease.

Teachers and students may be familiar with the book [The Cure](#) or the movie [Extraordinary Measures](#). Both of these did a tremendous job of telling the story of one man, one family, desperate to find a treatment for Pompe disease. I have chosen to only include these as extension activities, once the students have learned about the development of enzyme replacement therapy (Myozyme). Certainly, if people are more aware of Pompe disease, the efforts of the Pompe community are strengthened, so in that regard the book and movie are successes. Since they are essentially only one side of the story however, they lack balance. As a resource for the teacher before teaching this unit, both can be very useful, particularly the book, keeping in mind it is one person's perspective. Myozyme did not come into use based on the work of one scientist, father, or biotechnology company. It is the success of many individuals throughout the world contributing to the body of knowledge in a race against the clock.

# Tips about this Curriculum



**Lesson Plan Format:** All lessons in this curriculum unit are formatted in the same manner. In each lesson you will find the following components:

**KEY QUESTION(S):** Identifies key questions the lesson will explore.

**OVERALL TIME ESTIMATE:** Indicates total amount of time needed for the lesson, including advanced preparation.

**LEARNING STYLES:** Visual, auditory, and/or kinesthetic.

**VOCABULARY:** Lists key vocabulary terms used and defined in the lesson. Also collected in master vocabulary list.

**LESSON SUMMARY:** Provides a 1-2 sentence summary of what the lesson will cover and how this content will be covered. Also collected in one list.

**STUDENT LEARNING OBJECTIVES:** Focuses on what students will know, feel, or be able to do at the conclusion of the lesson.

**STANDARDS:** Specific state benchmarks addressed in the lesson. Also collected in one list.

**MATERIALS:** Items needed to complete the lesson. Number required for different types of grouping formats (Per class, Per group of 3-4 students, Per pair, Per student) is also indicated.

**BACKGROUND INFORMATION:** Provides accurate, up-to-date information from reliable sources about the lesson topic.

**ADVANCE PREPARATION:** This section explains what needs to be done to get ready for the lesson.

**PROCEDURE WITH TIME ESTIMATES:** The procedure details the steps of implementation with suggested time estimates. The times will likely vary depending on the class.

**ASSESSMENT SUGGESTIONS:** Formative assessment suggestions have been given. Additionally, there is a brief summative assessment (pre/post test) that can be given. Teachers should feel free to create additional formative and summative assessment pieces.

**EXTENSIONS: (ACTIVITIES/LITERATURE)** There are many activities and reading sources available to augment and enhance the curriculum. They have been included. If you find additional ones that should be added, please let us know.

**RESOURCES/REFERENCES:** This curriculum is based heavily on primary sources. As resources and references have been used in a lesson, their complete citation is included as well as a web link if available. All references and resources are also collected in one list.

**STUDENT PAGES:** Worksheets and handouts to be copied and distributed to the students.

**TEACHER MASTERS:** Versions of the student pages with answers or the activity materials for preparation.

**Collaborative Learning:** The lessons in this curriculum have been developed to include many collaborative learning opportunities. Rather than presenting information in lecture format and teacher driven, the activities involve the students in a more engaged manner. For classrooms not accustomed to using collaborative learning strategies, have patience. It can be difficult to communicate instructions, particularly for students who are visual learners. For these students, use of visual clues such as flowcharts and graphics can help them understand how they are to move to different groups.

**Groups:** Most of the lessons are carried out in groups. While it is not necessary for students to remain in the same groups the entire unit, if they work well together, it may foster students to think deeper as they are comfortable with their teammates and willing to ask questions of each other.

**Inquiry-based:** The lessons in the curriculum invite students to be engaged and ask questions. They work through background information in a guided fashion, but are challenged to think beyond what they have read or done. The teacher serves as the facilitator in these activities, not the deliverer of information.

**Technology:** Lessons have been written to be mindful of varying availability of technology in schools and homes. Some of the lessons would be very well suited to online environments and if your students are able, you might wish to engage in some of the technology modifications.

**Content:** Often we teach in a manner that is very content heavy. With high-stakes testing the norm, students are pushed to memorize and regurgitate numerous isolated facts. There is so much content that must be covered in a biology class, for example, that often it is difficult to synthesize those discrete facts into a compelling context or a story. This unit provides that opportunity: to take concepts learned such as muscles have a lot of glycogen or DNA codes for RNA, and put them in the context of disease. The lessons aren't designed to teach students what lysosomes do or transcription is, but rather *why* these ideas are important and *how* they can be used by researchers.

**Implementation notes:** This curriculum should be modified and adapted to suit the needs of the teacher and students. To help make implementation easier in this first draft, notes have been included in lessons as needed.

**Extensions:** For those teaching the AP Biology curriculum, bacterial transformation is a required laboratory. While bacterial transformation can be very useful in the research lab and certainly it helps students understand how bacteria gain new traits, it is also somewhat abstract from their daily lives. Other than the concept of antibiotic resistance (which is extremely important for students to understand), there is not a great deal of application for them. Likewise, in this unit, students may have difficulty conceptualizing how a hamster ovary cell can produce an enzyme for human use. Bacterial transformation can be used to model this process: transfection and cell culture in a pharmaceutical manufacturing facility is analogous to transformation and overnight bacterial cultures in an academic laboratory. For example, students can complete the BioRad pGLO bacterial transformation, pick and grow a transformed colony, purify the protein using column chromatography, and even perform a Western blot to confirm the protein of interest is present and test for purity, much like quality assurance in a pharmaceutical company.

Additional activities including BLAST, pedigree analysis using restriction digestion and gel electrophoresis, and 3D protein modeling can be developed and included with the Pompe theme.

**Science Subject:** Biology

**Grade and ability level:** 9-12 students in advanced biology

**Science concepts:** enzymes, DNA, mutations, replication, transcription, translation, protein structure, protein function, genetics, homozygous, heterozygous, dominant, recessive, cell structure

### Icon Key



Extension



Teacher Pages



Student Reading



Teacher Answer Key



Student Handout/  
Worksheets

# Lesson Summaries

## **LESSON ONE:**

### Looking Through a Father's Eyes

A first person story is presented to the students to hook their interest in the disease. Using a jigsaw approach, students will learn about the fundamentals of Pompe disease and share information during a whole class discussion. This activity sets the stage for further investigation of Pompe disease specifically, but more generally the steps of translational research. This lesson also challenges students to think in reverse. We often know the symptoms and pathology of diseases and perform a differential diagnosis to determine which disease fits the symptoms, then proceeding with treatment accordingly. In this lesson, students will understand the basis of the disease, know what cellular structures are affected, deduce the symptoms and propose treatment.

## **LESSON TWO:**

### The Road to Treatment

Working in groups, students will read Pompe fact cards and use text clues to sequence the events in the discovery and treatment of Pompe disease. This lesson illustrates scientific discovery as a collaborative effort of many individuals building on prior knowledge and developing unique ideas to explore.

## **LESSON THREE:**

### Pompe Journal Club

Using a reading guide, students work in pairs to read a journal article and share their interpretations during a whole class activity. After completing the introductory reading activity, students utilize a reading guide to work independently to read and identify key information before sharing their understanding with others in a small group. If time allows, students translate their paper into a poster to share with classmates during a gallery walk and subsequent poster presentation.

## **LESSON FOUR:**

### From DNA Structure to Function

With a commercially available kit from Science Take-Out, students step through the process of transcribing and translating DNA sequence. Using the student worksheet provided here, student then consider how the acid alphasglucosidase gene is affected by mutations and how the change in structure affects the function of the enzyme.

## **LESSON FIVE:**

### Putting it All Together

Students perform a colorimetric assay on patient samples to determine % activity of GAA. They then perform a confirmatory test by comparing the patient DNA sequence to the reference and identifying any mutations. They will transcribe their sequence into mRNA and translate into amino acids. Once they have their amino acid sequence, they will use chenille stems and beads to create the amino acid chain and fold their protein. Comparing the mutant protein to the normal protein, students will infer the functionality of the mutant protein and predict the form of Pompe disease.

## **LESSON SIX:**

### Grand Rounds

Building on the previous activity, students are given the case report for their patient. They will present a summary of the case along with the lab results they found in a grand rounds fashion. This activity allows students the opportunity to read actual patient cases and work together to summarize the findings. When they present their summary to the class, each member of the group is responsible for presenting part of the case.

## **LESSON SEVEN:**

### Exploring Gene Therapy Clinical Trials through Webquest and Role Play

In this lesson, students will learn about the benefits, dangers, and ethical dilemmas associated with gene therapy clinical trials through a webquest. They will then participate in a role play, depicting various stakeholders at an RAC meeting to decide the fate of a gene therapy trial. This activity serves as a wrap-up to the entire unit.

## **LESSON EIGHT:**

### Extraordinary Measures video guide

After students have a deep understanding of the science of Pompe disease, students view the movie *Extraordinary Measures*, based on the book *The Cure* which tells the story of of the Crowley family and their battle against Pompe disease. Students complete a movie guide during the film and consider the accuracy of the Hollywood depiction drawing from their scientific knowledge of Pompe disease.



# Lesson Sequencing Guide

Since each classroom teacher knows his or her students best, the teacher should decide the sequencing of lessons. Below is a suggested pacing guide that can be used when planning to use this curriculum.

	Day 1	Day 2	Day 3	Day 4	Day 5
<b>Week 1</b>	<b>Lesson 1</b> Looking Through a Father's Eyes (45 minutes)	<b>Lesson 2</b> The Road to Treatment (30 minutes)	<sup>A</sup> <b>Lesson 3</b> Pompe Journal Club (45 minutes)	<sup>A</sup> <b>Lesson 3</b> Pompe Journal Club (45 minutes)	<sup>A</sup> <b>Lesson 3</b> Pompe Journal Club (45 minutes)
<b>Week 2</b>	<b>Lesson 4</b> From DNA Structure to Function (45 minutes)	<b>Lesson 5</b> Putting it All Together (45 minutes)	<b>Lesson 6</b> Grand Rounds (45 minutes)	<sup>B</sup> <b>Lesson 7</b> Exploring Gene Therapy Clinical Trials through Webquest and Role Play (45 minutes)	<b>Lesson 7</b> Exploring Gene Therapy Clinical Trials through Webquest and Role Play (30 minutes)
<b>Week 3</b>	<b>Lesson 7</b> Exploring Gene Therapy Clinical Trials through Webquest and Role Play (45 minutes)	<sup>C</sup> <b>Lesson 8</b> Extension Extraordinary Measures	<sup>C</sup> <b>Lesson 8</b> Extension Extraordinary Measures	<sup>C</sup> <b>Lesson 8</b> Extension Extraordinary Measures	

<sup>A</sup> Lesson 3 – Journal Club can be adapted for use outside of the classroom period with readings for homework and/or discussions taking place through web-based platforms

<sup>B</sup> Lesson 7 – In preparation for the Role Play activity, students should understand gene therapy. The webquest can be performed during classroom time in a computer lab, or assigned for homework.

<sup>C</sup> Lesson 8 – The movie can be shown after school, during lunch or as an in-class field trip. (Movie run time is 106 minutes.)

# Vocabulary

**Deletion:** removal of one or more nucleotides from a DNA sequence, which may alter the reading frame

**DNA:** Deoxyribonucleic acid is a nucleic acid containing the genetic instructions used in the development and functioning of all known living organisms.

**Enzyme:** Enzymes are proteins that catalyze (i.e., increase the rates of) chemical reactions. Almost all chemical reactions in a biological cell need enzymes in order to occur at rates sufficient for life.

**Enzyme replacement therapy (ERT):** a medical treatment replacing an enzyme in patients in whom that particular enzyme is deficient or absent. Usually this is done by giving the patient an intravenous (IV) infusion containing the enzyme. Enzyme replacement therapy does not “treat” the underlying disease, only the symptoms.

**Genotype:** The genotype is the genetic makeup of a cell, an organism, or an individual (i.e. the specific allele makeup of the individual). The genotype of an organism is the inherited instructions it carries within its genetic code.

**Grand rounds** are an important teaching tool and ritual of medical education and inpatient care, consisting of presenting the medical problems and treatment of a particular patient to an audience consisting of doctors, residents and medical students. The patient was traditionally present for the round and would answer questions; grand rounds have evolved with most sessions now rarely having a patient present and being more like lectures.

**Insertion:** addition of one or more nucleotides in a DNA sequence, which may alter the reading frame

**Lysosome:** Lysosomes are cellular organelles that contain acidic digestive enzymes to break down waste materials and cellular debris.

**Missense:** generally a single nucleotide change in the protein coding region that results in a stop codon, causing the protein to be truncated

**Nonsense:** generally a single nucleotide change in the protein coding region that results in a different amino acid

**Peer-review** is the act of having another writer read what you have written and respond in terms of its effectiveness. This reader attempts to identify the writing’s strengths and weaknesses, particularly reading

how sound the science is, and then suggests strategies for revising it. The hope is that not only will the specific piece of writing be improved, but that future writing attempts will also be more successful. Peer-review happens with all types of writing, at any stage of the process, and with all levels of writers.

**Pharmacological chaperone:** Small molecules that selectively bind to and stabilize proteins in cells, leading to improved protein folding and trafficking, and increased activity.

**Phenotype:** An organism’s observable characteristics or traits. Phenotypes result from the expression of an organism’s genes as well as the influence of environmental factors and the interactions between the two.

**Pompe disease:** Pompe disease is an inherited disorder caused by the buildup of a complex sugar called glycogen in the body’s cells. The accumulation of glycogen in certain organs and tissues, especially muscles, impairs their ability to function normally. There are three types of Pompe disease, which differ in severity and the age at which they appear. These types are known as classic infantile-onset, non-classic infantile-onset, and late-onset.

**Reading frame:** a way of breaking a sequence of nucleotides in DNA or RNA into three letter codons, resulting in a possibility of three reading frames in mRNA and six in double-stranded DNA (since have forward and reverse).

**RNA:** Ribonucleic acid is one of the three major macromolecules (along with DNA and proteins) that are essential for all known forms of life. Like DNA, RNA is made up of a long chain of components called nucleotides. Each nucleotide consists of a nucleobase, a ribose sugar, and a phosphate group. RNA directs the synthesis of proteins.

**Transcription:** DNA → RNA; During transcription, a DNA sequence is read by an RNA polymerase, which produces a complementary, antiparallel RNA strand. The RNA complement includes uracil (U) in all instances where thymine (T) would have occurred in a DNA complement.

**Translation:** RNA → Protein; In translation, messenger RNA (mRNA) produced by transcription is decoded by the ribosome to produce a specific amino acid chain, or polypeptide, that will later fold into an active protein.

## Next Generation Sunshine State Standards – Science (Florida)

Benchmark	Lesson							
	1	2	3	4	5	6	7	8
<p><b>SC.912.L.14.6</b>                      Explain the significance of genetic factors, environmental factors, and pathogenic agents to health from the perspectives of both individual and public health.</p>	X		X	X	X	X	X	X
<p><b>SC.912.L.14.19</b>                      Explain the physiology of skeletal muscle.</p>	X							X
<p><b>SC.912.L.14.20</b>                      Identify the major muscles of the human on a model or diagram.</p>	X							
<p><b>SC.912.L.16.2</b>                      Discuss observed inheritance patterns caused by various modes of inheritance, including dominant, recessive, codominant, sex-linked, polygenic, and multiple alleles.</p>	X			X	X	X		X
<p><b>SC.912.L.16.3</b>                      Describe the basic process of DNA replication and how it relates to the transmission and conservation of the genetic information.</p>	X			X	X			
<p><b>SC.912.L.16.4</b>                      Explain how mutations in the DNA sequence may or may not result in phenotypic change. Explain how mutations in gametes may result in phenotypic changes in offspring.</p>				X	X	X		
<p><b>SC.912.L.16.5</b>                      Explain the basic processes of transcription and translation, and how they result in the expression of genes.</p>				X	X	X		
<p><b>SC.912.L.16.7</b>                      Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology.</p>							X	
<p><b>SC.912.L.16.9</b>                      Explain how and why the genetic code is universal and is common to almost all organisms.</p>			X	X	X			
<p><b>SC.912.L.16.10</b>                      Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.</p>		X						X
<p><b>SC.912.L.16.12</b>                      Describe how basic DNA technology (restriction digestion by endonucleases, gel electrophoresis, polymerase chain reaction, ligation, and transformation) is used to construct recombinant DNA molecules (DNA cloning).</p>							X	

## Next Generation Sunshine State Standards – Science (cont.)

Benchmark	Lesson							
	1	2	3	4	5	6	7	8
<p><b>SC.912.L.18.1</b> Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules.</p>	X	X	X	X	X	X	X	
<p><b>SC.912.L.18.2</b> Describe the important structural characteristics of monosaccharides, disaccharides, and polysaccharides and explain the functions of carbohydrates in living things.</p>	X							X
<p><b>SC.912.L.18.4</b> Describe the structures of proteins and amino acids. Explain the functions of proteins in living organisms. Identify some reactions that amino acids undergo. Relate the structure and function of enzymes.</p>	X			X	X	X		X
<p><b>SC.912.L.18.11</b> Explain the role of enzymes as catalysts that lower the activation energy of biochemical reactions. Identify factors, such as pH and temperature, and their effect on enzyme activity.</p>	X			X	X	X		
<p><b>SC.912.N.1.1</b> Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and earth/space science, and do the following:</p> <ol style="list-style-type: none"> <li>pose questions about the natural world,</li> <li>conduct systematic observations,</li> <li>examine books and other sources of information to see what is already known,</li> <li>review what is known in light of empirical evidence,</li> <li>plan investigations,</li> <li>use tools to gather, analyze, and interpret data,</li> <li>pose answers, explanations, or descriptions of events,</li> <li>generate explanations that explicate or describe natural phenomena (inferences),</li> <li>use appropriate evidence and reasoning to justify these explanations to others,</li> <li>communicate results of scientific investigations, and</li> <li>evaluate the merits of the explanations produced by others.</li> </ol>		X						X
<p><b>SC.912.N.1.2</b> Describe and explain what characterizes science and its methods.</p>		X	X					X
<p><b>SC.912.N.1.3</b> Recognize that the strength or usefulness of a scientific claim is evaluated through scientific argumentation, which depends on critical and logical thinking, and the active consideration of alternative scientific explanations to explain the data presented.</p>		X	X				X	X

## Next Generation Sunshine State Standards – Science (cont.)

Benchmark	Lesson							
	1	2	3	4	5	6	7	8
<p><b>SC.912.N.1.5</b> Describe and provide examples of how similar investigations conducted in many parts of the world result in the same outcome.</p>		X	X					X
<p><b>SC.912.N.1.6</b> Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied.</p>	X			X	X			X
<p><b>SC.912.N.1.7</b> Recognize the role of creativity in constructing scientific questions, methods and explanations.</p>		X						X
<p><b>SC.912.N.2.4</b> Explain that scientific knowledge is both durable and robust and open to change. Scientific knowledge can change because it is often examined and re-examined by new investigations and scientific argumentation. Because of these frequent examinations, scientific knowledge becomes stronger, leading to its durability.</p>		X	X					X
<p><b>SC.912.N.2.5</b> Describe instances in which scientists' varied backgrounds, talents, interests, and goals influence the inferences and thus the explanations that they make about observations of natural phenomena and describe that competing interpretations (explanations) of scientists are a strength of science as they are a source of new, testable ideas that have the potential to add new evidence to support one or another of the explanations.</p>		X	X					X
<p><b>SC.912.N.3.5</b> Describe the function of models in science, and identify the wide range of models used in science.</p>				X	X			
<p><b>SC.912.N.4.1</b> Explain how scientific knowledge and reasoning provide an empirically-based perspective to inform society's decision making.</p>							X	X
<p><b>SC.912.N.4.2</b> Weigh the merits of alternative strategies for solving a specific societal problem by comparing a number of different costs and benefits, such as human, economic, and environmental.</p>							X	X

## Next Generation Science Standards

As *The Pompe Predicament* is written for high school students, only high school performance expectations are included. Some topics such as gene therapy (ML-LS4-5) and use of Punnett squares (MS-LS3-2) are included in the middle school performance expectations and therefore not listed as a high school performance expectation.

Performance Expectations: High School Life Science	Lesson							
	1	2	3	4	5	6	7	8
<b>HS-LS1-1</b> Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.	X							
<b>HS-LS1-2</b> Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms.	X		X	X	X	X		
<b>HS-LS1-3</b> Plan and conduct an investigation to provide evidence that feedback mechanisms maintain homeostasis.  Connection to the Nature of Science: Scientific Investigations Use a Variety of Methods <ul style="list-style-type: none"> <li>Scientific inquiry is characterized by a common set of values that include: logical thinking, precision, open-mindedness, objectivity, skepticism, replicability of results, and honest and ethical reporting of findings.</li> </ul>				X	X	X		
<b>HS-LS1-4</b> Use a model to illustrate the role of cellular division (mitosis) and differentiation in producing and maintaining complex organisms.			X	X	X	X	X	
<b>HS-LS3-1</b> Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.	X		X	X	X	X	X	X
<b>HS-LS3-2</b> Make and defend a claim based on evidence that inheritable genetic variations may result from: (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.	X		X	X	X	X	X	
<b>HS-LS3-3</b> Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population. [Clarification Statement: Emphasis is on the use of mathematics to describe the probability of traits as it relates to genetic and environmental								
Connections to Nature of Science: Science is a Human Endeavor <ul style="list-style-type: none"> <li>Technological advances have influenced the progress of science and science has influenced advances in technology.</li> <li>Science and engineering are influenced by society and society is influenced by science and engineering.</li> </ul>	X		X	X	X	X		

# Background Information

General background information is given here. More detail is provided in the individual lessons as needed as well in the student information in lesson one.

**P**ompe disease is a rare, fatal illness affecting the heart and muscles. Pompe disease is one of a family of 49 rare genetic disorders known as Lysosomal Storage Diseases or LSDs. Pompe disease is also known as Acid Maltase Deficiency or Glycogen Storage Disease type II. It is caused by a mutation in the gene that produces the enzyme acid alpha-glucosidase (GAA) (also known as acid maltase) which results in complete or partial deficiency of the lysosomal enzyme GAA. This enzyme is necessary to break down glycogen and to convert it into glucose. Without this enzyme, glycogen accumulates in the lysosomes and leads to severe muscle degradation. It predominately affects the heart, skeletal, and respiratory muscles of the patient where glycogen is abundant. As the lysosomes swell with the glycogen, they eventually cause the cells to break open, causing irreversible damage to the surrounding muscle tissue. Pompe disease is a recessive genetic disorder. Both parents must carry the gene for it to be transmitted to a child. Mutations in the gene determine when and how the active illness will appear. Studies indicate that Pompe disease occurs in 1 in 40,000 live births worldwide. The number of people with Pompe disease is estimated at approximately 5,000-10,000 patients. Pompe disease affects both men and women. There is no cure for Pompe disease. Most patients die from cardiac or respiratory failure.

Clinical forms of the disease vary according to the age of onset and percent of enzyme activity.

**THE EARLY ONSET FORM (infantile or classic):** Early onset is characterized by a complete deficiency of the enzyme. Most babies born with Pompe disease die from cardiac or respiratory complications before their first birthday. Symptoms appear almost immediately after birth. Babies often appear “floppy” due to muscle weakness. The heart and liver are enlarged. Respiratory problems occur and a ventilator will eventually be required to assist breathing. Many infant patients have enlarged tongues, which impacts feeding, swallowing, and weight gain. Regular development, such as rolling over, crawling, or sitting up, is delayed, if possible at all. The heart progressively thickens and enlarges and respiratory distress is common. Early onset patients may benefit from enzyme replacement therapy (ERT). In 2006, the Federal Drug Administration (FDA) approved Myozyme, an ERT made by the pharmaceutical company Genzyme. The ERT infuses a man-made version of the enzyme directly into the veins of patients.

**THE DELAYED ONSET FORM (subdivided into juvenile and late onset):** Delayed onset patients produce a minimal amount of enzyme. Progression and severity of the disease is probably attributable to the amount of enzyme produced and to the age of onset of symptoms. Glycogen build up is not as rapid as in the infantile form but the disease is progressive and can greatly decrease the life span of the afflicted person. In the delayed onset form deterioration of muscle is mainly confined to the skeletal muscles, the diaphragm, the limb-girdle, and the trunk. Respiratory complications are the main cause of death. Delayed onset patients that present symptoms early in life are usually more severely affected and rarely survive past the second or third decade of life. Patients that experience onset later in life generally progress at a slower pace. In advanced cases, the diaphragm will become paralyzed. Infections such as bronchitis and pneumonia frequently occur. Patients with difficulty chewing and swallowing may require a feeding tube to keep their weight stable. Temporary respirators are often used at night to keep oxygen levels stable and allow the patient to have a restful night's sleep. Patients with late onset deteriorate at varying rates, depending upon the age of onset and the progression of the disease. All of these conditions worsen over time. In 2010, ERT was approved for use in late onset patients and Myozyme was repackaged as Lumizyme.

## Background Information (continued...)

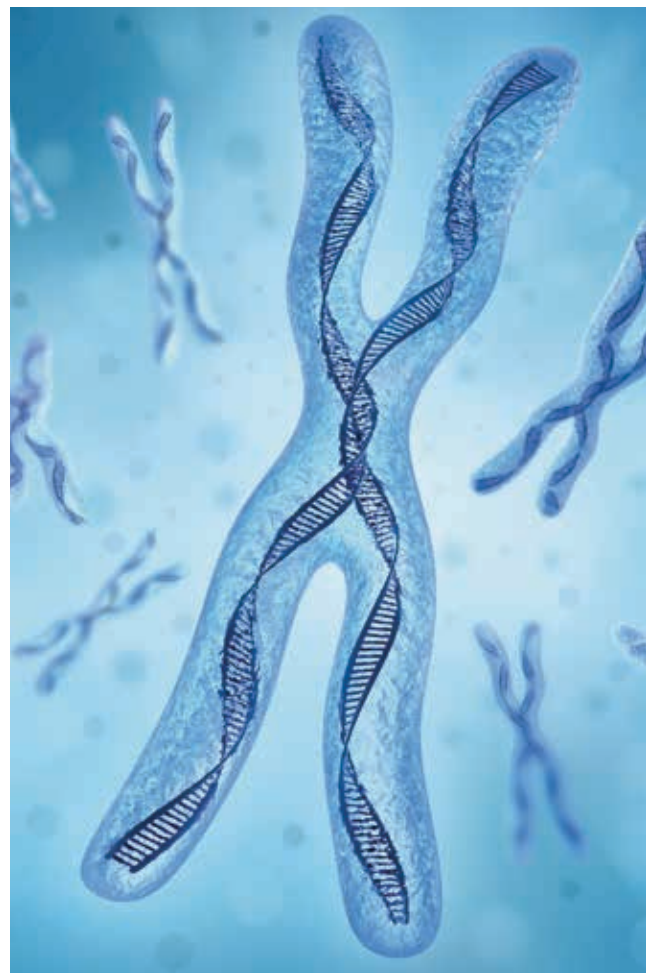
### Patient Care

Research is ongoing for treatment of Pompe disease. Genzyme's ERT is the only current (2011) available therapy for Pompe disease. It slows the progression of the illness, but does not cure it. The ERT is given through infusion—intravenously—much like chemotherapy is given to cancer patients. The ERT has been proven to slow progression of the illness, reduce heart size, improve both heart and muscle function, and reduce glycogen accumulation in the cells. Pompe patients who benefit from the ERT require an infusion every two weeks for the duration of their lives. The cost of the ERT is approximately \$300,000 per year. Other pharmaceutical companies are beginning clinical trials for oral chaperones that would augment current ERT and potentially increase the amount of the enzyme that enter the lysosome.

### Enzyme Replacement Therapy

In 1999 the first human clinical trials for Pompe disease with enzyme replacement therapy (ERT) began in the Netherlands with four infantile patients at Sophia Children's Hospital in Rotterdam. Six months later another clinical trial was started in the Netherlands with three delayed onset patients. At the same time Duke University Medical Center in the US initiated a new infantile clinical trial with three patients.

Genzyme Corporation, the sponsor of these first clinical trials, received FDA and EMEA approval for enzyme replacement therapy for infant and juvenile forms in 2006. Myozyme is the enzyme replacement therapy currently administered to these younger patients. Lumizyme was approved and administered for the first time in adult patients in 2010. ERT is currently the only treatment available to Pompe patients. Second generation ERT is in early stage clinical trials with the hope of increasing the amount of acid alpha-glucosidase that actually reaches the lysosomes.



### Gene Therapy

Gene therapy is considered the only hope for a cure for Pompe disease and other rare disorders. There are numerous laboratories working on gene therapy and clinical trials in process, but as yet, gene therapy is not commercially available.



# Looking through a Father's Eyes

# 1

## Vocabulary:

**Pompe disease:** Pompe disease is an inherited disorder caused by the buildup of a complex sugar called glycogen in the body's cells. The accumulation of glycogen in certain organs and tissues, especially muscles, impairs their ability to function normally. There are three types of Pompe disease, which differ in severity and the age at which

they appear. These types are known as classic infantile-onset, non-classic infantile-onset, and late-onset.

**Lysosome:** Lysosomes are cellular organelles that contain acidic digestive enzymes to break down waste materials and cellular debris.

**Enzyme:** Enzymes are proteins that catalyze (i.e., in-

crease the rates of) chemical reactions. Almost all chemical reactions in a biological cell need enzymes in order to occur at rates sufficient for life.

**DNA:** Deoxyribonucleic acid is a nucleic acid containing the genetic instructions used in the development and functioning of all known living organisms.

## ? KEY QUESTION(S):

- What is Pompe disease?
- What is the fundamental biology of the disease?

## 🕒 TIME ESTIMATE:

- Advanced Preparation: 60 minutes (30 minutes to assemble section packets; 30 minutes background reading)
- Student Procedure: 45-60 minutes

## 🎧 LEARNING STYLES:

- Visual and auditory

## Lesson Summary:

A first person story is presented to the students to hook their interest in the disease. Using a jigsaw approach, students will learn about the fundamentals of Pompe disease and share information during a whole class discussion. This activity sets the stage for further investigation of Pompe disease specifically, but more generally the steps of translational research. This lesson also challenges students to think in reverse. We often know the symptoms and pathology of diseases and perform a differential diagnosis to determine which disease fits the symptoms, then proceeding with treatment accordingly. In this lesson, students will understand the basis of the disease, know what cellular structures are affected, deduce the symptoms and propose treatment.

## Student Learning Objectives:

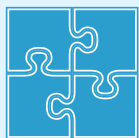
The student will be able to...

1. Describe an enzyme.
2. Explain the role of an enzyme in the human body.
3. Define gene and understand how a gene relates to a protein
4. Explain where a lysosome is located and its role in the function of a cell
5. Explain the genetics of Pompe disease
6. Associate disease pathology with symptoms
7. Suggest treatment methods for Pompe disease

## Standards:

See table on page 11-13.

SC.912.L.14.6	SC.912.L.14.20	SC.912.L.16.3	SC.912.L.18.2	SC.912.L.18.11
SC.912.L.14.19	SC.912.L.16.2	SC.912.L.18.1	SC.912.L.18.4	SC.912.N.1.6



For students and classrooms unaccustomed to jigsaws, it can seem a bit confusing and chaotic. Have patience. Collaborative learning experiences are a valuable part of scientific discovery.

## Materials:

- 1 copy of *Teacher Pages: Jigsaw – Enzymes – Lysosomes*
- 1 copy of *Teacher Pages: Section Name Cards*
- Envelopes or clips (for keeping information pages together)
- 1 copy of *Student Worksheet: Looking Through a Father's Eyes* per student

## Background Information:

Teachers are encouraged to read the student information (four sections: enzymes, molecular basics, genetics, and lysosome) prior to the activity. This activity specifically focuses on the fundamentals of Pompe disease to help students understand what it is so they can think further about how it might be diagnosed and treated.

## Advance Preparation:

1. Make section cards: *Teacher Pages: Section Name Cards*, laminate if desired, and cut into individual cards.
2. Make information section packets: Copy *Teacher Pages: Jigsaw – Enzymes – Lysosomes*. Laminate if desired. Cut into smaller reading sections by cutting between paragraphs. Place all slips for a section in an envelope or clip together.
3. Make 1 copy of *Student Worksheet: Looking Through a Father's Eyes* for each student.
4. Make overhead or prepare paper copies of JAMA overview of basic science research <http://jama.ama-assn.org/content/287/13/1754.full.pdf>
5. If having a student read Calum's story, make an extra copy for the student to read from.

## Procedure and Discussion Questions with Time Estimates:

1. **(2 min)** Read aloud or ask a student to read Calum's story aloud.
2. **(5 min)** Ask the students to share their thoughts on the story. Record their comments/questions on the board or other medium that can be referenced throughout the unit (i.e., flip chart paper, transparency, Smartboard, etc.) Students may note the year 1993 and suggest that surely there is a cure or at least a treatment now. This is also a good time to assess prior knowledge (particularly related to the movie *Extraordinary Measures*).
3. **(2 min)** Tell the students they will have a chance to learn more about Pompe disease over the course of the next several classes. Their first task is to learn the fundamentals of the disease (the basic science).
4. **(5 min)** Project the JAMA overview of basic science research. Allow students to read and summarize the main points. <http://jama.ama-assn.org/content/287/13/1754.full.pdf>  
**Implementation note:** *Optional stopping point and/or inclusion of an enzyme activity.*
5. **(5 min)** Have students assemble into groups of 4. This is their home group, so encourage them to remember the members of their home group. For a class with extra students, have them join to make groups of 5 rather than have a group without a member which would put extra burden on the smaller group to read more.
  - Once settled, give each member a card with one of the following section names: Enzymes, Molecular Basics, Genetics, or Lysosome. See *Teacher Pages: Section Name Cards*.

Group	Member A	Member B	Member C	Member D
1	Enzymes	Molecular Basics	Genetics	Lysosome
2	Enzymes	Molecular Basics	Genetics	Lysosome
3	Enzymes	Molecular Basics	Genetics	Lysosome
4	Enzymes	Molecular Basics	Genetics	Lysosome
5	Enzymes	Molecular Basics	Genetics	Lysosome
6	Enzymes	Molecular Basics	Genetics	Lysosome
7	Enzymes	Molecular Basics	Genetics	Lysosome
8	Enzymes	Molecular Basics	Genetics	Lysosome

6. Ask students to regroup according to their section name, forming four large groups. *\*You can allow students to stay in these large groups, or divide them in half yielding 2 Enzyme sections, 2 Molecular Basics sections, 2 Genetics sections, and 2 Lysosome sections. This option requires producing two copies of each section information packet.*



### EXTENSIONS:

An enzyme lab to demonstrate enzyme activity

Peroxide activity:  
[http://www.biologycorner.com/worksheets/enzyme\\_lab.html](http://www.biologycorner.com/worksheets/enzyme_lab.html)

Pineapple juice gelatin lab:  
[http://mdk12.org/instruction/curriculum/hsa/biology/enzyme\\_activity/teachers\\_guide\\_engagement2.html](http://mdk12.org/instruction/curriculum/hsa/biology/enzyme_activity/teachers_guide_engagement2.html)

Section Name	Group	Member #
Enzymes	Group 1-8	Member A
Molecular Basics	Group 1-8	Member B
Genetics	Group 1-8	Member C
Lysosome	Group 1-8	Member D

- Distribute information packets to each section.
- Distribute *Student Worksheet: Looking Through a Father's Eyes* to each student.
- (10 min)** Have students remove and equally distribute the information slips from the envelope, read their slip(s), and share with the other members of their section. Encourage students to take notes and summarize their section to share back in their home group. Move around the groups to ensure understanding.
- Ask students to put their information slips back in the envelope or clip. Have one member return the envelope to the front of the room while the groups redistribute back to their home group.

Group	Member A	Member B	Member C	Member D
1	Enzymes	Molecular Basics	Genetics	Lysosome
2	Enzymes	Molecular Basics	Genetics	Lysosome
3	Enzymes	Molecular Basics	Genetics	Lysosome
4	Enzymes	Molecular Basics	Genetics	Lysosome
5	Enzymes	Molecular Basics	Genetics	Lysosome
6	Enzymes	Molecular Basics	Genetics	Lysosome
7	Enzymes	Molecular Basics	Genetics	Lysosome
8	Enzymes	Molecular Basics	Genetics	Lysosome

- (10 min)** Once back in their home groups, the students should each take a turn sharing what they learned about Pompe disease in their section group. Again, move around the groups to ensure understanding. They can use the *Student Worksheet: Looking Through a Father's Eyes* to guide their discussion.
- (10-15 min)** Drawing from the student comments/questions from the beginning and using the *Student Worksheet: Looking Through a Father's Eyes*, call on groups to answer the questions on the worksheet aloud. Use the *Teacher Answer Key* to check for understanding. Encourage other groups to share their interpretations as well. Clarify uncertain concepts. Students may need to be guided to answer questions 5 – 7 on the worksheet or these may be left for students to complete on their own. See the *Teacher Answer Key* for suggested questions to pose.
- Conclude by telling the students they should now understand the cause of the disease and have suggested symptoms and treatment. This is the beginning of the basic science of translational research, the process of producing a therapeutic product. Next they will look closer at the history and future of Pompe disease.

## Assessment Suggestions:

- Student worksheet can be checked for completion.

## RESOURCES/REFERENCES:

Background information: Online Mendelian Inheritance of Man <http://omim.org/entry/232300>

Background information: <http://www.amda-pompe.org/index.php/main/about/amd/>

Background information: <http://www.pompe.com/en/>

Background information and informational materials: <http://www.pompe.org.uk/>

Calum's story: <http://pompestory.blogspot.com/2009/04/calum.html>

Additional patient stories: <http://www.amda-pompe.org/index.php/main/patients/>

Section information:

*A Guide for Families* by Kevin O'Donnell, 1994 [http://www.pompe.org.uk/images/stories/guide\\_for\\_families.pdf](http://www.pompe.org.uk/images/stories/guide_for_families.pdf)

Rare Disease Day: <http://www.rarediseaseday.org/>



## Calum's Story

WRITTEN BY KEVIN O'DONNELL

It was Friday, May 28, 1993 and one of the happiest days of my life. I was driving across the border from England into Scotland, to a new home in Edinburgh – the city I had always wanted to live in – and to my dream job, running my own molecular biology laboratory. Best of all, I had my family in the car with me – my wife Elaine and our two-month old baby son, Calum. Life was sweet. Yet within a few short months that sweetness turned to dust in my mouth.

Everything was fine for a couple of months. I busied myself with settling in to my new job, while Elaine and Calum went house-hunting. Then Calum seemed to be getting a lot of colds and sniffles, which took longer and longer to go away. It never occurred to us for one moment that something could be seriously wrong. Even when, at six months old, Calum was hospitalized with pneumonia we didn't think it was anything other than a temporary blip. In hindsight we were incredibly naive – but what does anyone know with their first child?

An x-ray showed an enlarged heart and, even then, we didn't think anything other than "So he shouldn't play rough sports? Guess he'll just have to be geeky like his dad!" Unknown to us though, alarm bells were beginning to ring at the hospital and blood tests were taken "Just routine, nothing to be concerned about..." And we weren't concerned. We found a house (next to a park – and a school – perfect!) and started fixing it up prior to moving in.

Then we got the news that brought our world crashing down. On a follow-up hospital visit we were told that the blood tests showed Calum had something called Pompe disease which was a type of glycogen storage disease. It was untreatable and fatal; children with this disease did not usually live beyond their first birthday.

We just couldn't believe it. We thought that there must have been some terrible mistake. Our beautiful child, the light of our lives, going to die? How could such a thing be possible? And whoever heard of an untreatable disease in this day and age – those doctors really needed to keep up with what was happening in the world of medicine!

For the next two weeks we frantically found out everything we could about Pompe disease and what little information there was available was not good. Everything confirmed what the hospital had told us; there was no hope. None. While we were still in shock, Calum declined quite rapidly and he went back into the hospital. He died at the Hospital in Edinburgh, on November 18, 1993, just two weeks after we were given the diagnosis.

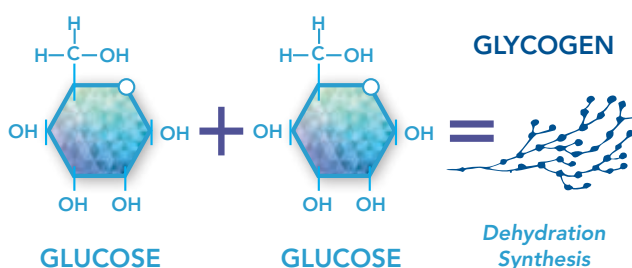
[http://pompestory.blogspot.com/2009\\_04\\_01\\_archive.html](http://pompestory.blogspot.com/2009_04_01_archive.html)



## Jigsaw – Enzymes

**A** Pompe disease is caused by a defective enzyme, acid alpha-glucosidase (pronounced “AL-fa glue-CO-sih-days” and often abbreviated GAA). But what is an enzyme and why are they so important? In your body, right now, thousands of different chemical reactions are taking place. The chemical reactions that take place in the body are controlled by enzymes. Of direct interest to us, are the reactions concerning sugar.

**B** Small units of sugar, glucose, are being bound together to make glycogen to use for energy later. In turn, when energy is needed, glycogen is broken down into small sugar molecules. Yet, if you take a lump of sugar and place it on your kitchen table you will wait a very long time before it turns into either energy or glycogen. You need a *catalyst*, something which will help the sugar to change into something else. In the body, **enzymes** are the catalysts which make reactions like this happen.



**C** There are enzymes for all sorts of purposes: one enzyme may catalyze the formation of glycogen and another may break it down. Each one of the thousands of reactions which make up a human being is catalyzed by an enzyme. This is under strict control, otherwise there would be chaos. The control is done by the body being divided into compartments (of which we see more later) and by the activity of the enzymes being regulated by... other enzymes. You will gather from this that enzymes determine what we are and that living things are extremely complicated.

**D** So, having explained what they do, what are enzymes? **Enzymes are proteins, made up of a chain of building blocks called amino acids.** There are twenty different amino acids and an almost infinite number of ways in which they can be arranged to make different proteins. Not all proteins are enzymes – some proteins are part of the body’s structure. For example, muscle is largely protein, as are fingernails and hair. The function of a protein is determined by which amino acids make it up and how they are joined together. This is because although proteins are made up of a single long chain of amino acids, they do not exist as a long piece of string.

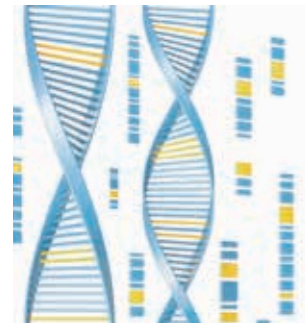
**E** If a protein was big enough to see with the naked eye, it would look more like a tangled piece of string. This is because certain amino acids cause kinks in the chain, causing it to double back on itself or go at an angle. Also, some amino acids will form bonds with others they find themselves close to due to the kinks and bends in the chain. So, although it might look like a tangled piece of string, the shape of proteins is not random. Every time the body makes a copy of a particular protein it has exactly the same shape. This is important.

**F** **It is this specific 3-dimensional (3D) shape of enzymes that determines how they interact with the chemicals that make up the body.** For example, an enzyme with one shape may hold two sugar molecules together, while another enzyme may break them apart. This is a simplified statement, but that is essentially how enzymes work. If an enzyme for some reason has a wrong amino acid in the chain, then its shape will be different and it may not function properly. That is what happens in metabolic diseases such as Pompe disease.



## Jigsaw – Molecular Basics

**A** **DNA (deoxyribonucleic acid) is the chemical in which all of our genetic information is stored.** A complete copy of our DNA is in every cell in our body (except mature red blood cells which lack a nucleus). Like proteins, it is made up a series of building blocks. In the case of DNA however, **there are only four building blocks called nucleotides: adenine, thymine, cytosine and guanine.** We will refer to them as A, T, C and G. These are the letters in which the genetic code is written. These letters join together to make extremely long stretches of DNA called chromosomes. The letters are not joined together at random. They make up a series of 'words'. **Each 'word' is made up of three letters, and signifies (or 'codes for') a specific amino acid.** Each amino acid has at least one three letter 'word' coding for it, some have more than one. This is the genetic code.



**B** Some 'words' (or **codons** as they are called) do not code for amino acids but are punctuation marks. They tell the machinery of the body which reads the genetic code to either start or stop reading. **The 'words' in between a start and stop signal code for amino acids which, when joined together, will make up a single protein.** This unit of information is called a **gene**. This is the way in which the information in DNA is used to make each protein and therefore all living things.

**C** When cells grow, they divide into two, to make new cells. When this happens, the entire genome (three billion nucleotides) must be copied (replicated). In each human, there are around 25,000 genes, each coding for thousands of nucleotides and hundreds of amino acids. Occasionally mistakes are made and one nucleotide letter is substituted for another. When a gene which contains a wrong letter is read, the wrong amino acid is placed in the protein. This can give the protein a different 3D shape.

**D** With Pompe disease, we are concerned with the structure and function of the GAA protein which is an important enzyme. A misfolded protein may result in reduced function and performance of the enzyme. This is particularly damaging if the new 'word' formed with the wrong letter is the one which says 'stop reading', in which case a shortened enzyme is formed which will have no function at all. Also very damaging is when an extra base is inserted into the DNA sequence or one is deleted without being replaced. This means that from that point on, all the three letter 'words' will be wrong. For example, take AAT TTA GGC. If an extra C is inserted at the beginning, this will give CAA TTT AGG C. This is a completely different series of words giving a completely different, and possibly functionless, enzyme.

**E** **Changes in the DNA sequence are called mutations.** Most go undetected; however, some are harmful. **The DNA change may result in an enzyme produced which is not as good as the original one.** This disrupts the normal function of the cell and body systems and can lead to severe complications. In a single cell, mutations usually do not matter very much and would not even be noticed. The problems come when the cell in which the mutated DNA is present is a germ cell (sperm or egg) which goes to make up a new person. This means that every cell of that person has a faulty copy of DNA. Even then, this is not a disaster since DNA exists in the form of chromosomes. We each have two complete sets of chromosomes, one from each parent. So, if one set has a faulty gene, the copy of the gene from the other parent can be used instead.

**F** This is a great system, so good in fact that each of us has several faulty genes which are undetectable because we function perfectly well with the remaining functional copy. If we each have, say, 5 faulty genes out of 25,000, then the chances of meeting someone with the corresponding faulty genes are very small. That is why diseases like Pompe are so rare.

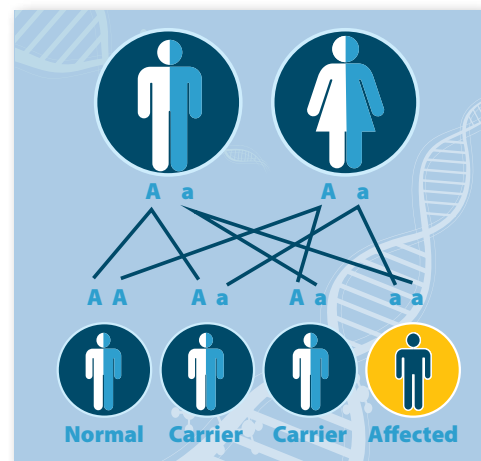


## Jigsaw – Genetics

**A** For parents of children with Pompe disease, each parent has one good gene and one faulty one (for the alpha-glucosidase enzyme GAA). They each pass on one gene to their children through random assortment. In the case of Pompe disease, **both parents have passed on the faulty gene, meaning that the child has no copy of the gene to make the functioning enzyme, leading to all the Pompe symptoms.**

**B** Pompe disease is an example of an autosomal recessive disorder. In order for symptoms of Pompe disease to present, an affected individual must have inherited a mutation from each parent. Pompe disease is quite rare; only 1 in 40,000 people are affected. Therefore, most individuals in the population do not have a mutation for Pompe, and their genotype is defined as AA. These individuals are referred to as **homozygous dominant**: they have the same genes on each chromosome and that gene corresponds to the dominant phenotype. In the example of Pompe disease, AA individuals have two normal functioning GAA genes.

**C** **Homozygous individuals have two copies of the same gene. Heterozygous individuals have different copies of the gene.** In a normal, non-affected individual, if a faulty GAA gene is present, it is masked by a functioning, normal gene (dominant). Let's define this person's genotype as Aa. A person with this genotype is considered a carrier since he or she carries a copy of the defective gene. So, unless symptoms are present, we assume a non-affected individual to be either AA (homozygous dominant) or Aa (heterozygous). Most heterozygous individuals do not know they carry the mutant gene until a family member presents with the disease. In order for symptoms to be present in a recessive disorder, the affected individual must have two faulty copies of the gene. In this case, two bad copies of the GAA gene. The genotype for this individual is written aa (**homozygous recessive**).



**D** So, if both parents are carriers, what is the likelihood of their children having Pompe disease? Using a Punnet square illustrates the possible genetic combinations. Remember, **A** indicates a normal gene for GAA; **a** indicates the mutated GAA gene. **The parents are both carriers with the genotype Aa.** We put one parent on the top of the square; the other parent on the left side. **The possible offspring combinations are denoted within the 4 squares: 1 in 4 (25%) possibility that the child will be affected (aa); 2 in 4 (50%) that the child will be a carrier (Aa); 1 in 4 (25%) that the child will have only normal genes (AA).** Since this is an autosomal disease, the mutation can be passed by either mother or father and all children, regardless of gender, have an equal chance of inheriting mutations.

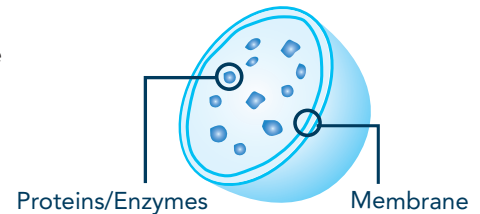
	<b>A</b>	<b>a</b>
<b>A</b>	<b>AA</b>	<b>Aa</b>
<b>a</b>	<b>Aa</b>	<b>aa</b>



# Jigsaw – Lysosomes

- A** The body is divided into tiny cells, each containing an entire copy of the DNA for the whole body. Not all of it is used in each cell. Liver cells read only the bits about liver cells, muscle cells only the bits about muscle cells and so on. That's why there are different types of cells in the body. Cells themselves are banded together to form organs. Just as the body is divided into organs, each with its own specific function, the **cells themselves are divided into compartments called organelles.**

## LYSOSOME STRUCTURE



- B** There are a number of different organelles in cells. Energy is produced in organelles called mitochondria; DNA, in the form of chromosomes, is kept in the nucleus; but our interest is in an organelle called the lysosome. Lysosomes are sacks (vesicles) of enzymes that have pinched off of the Golgi apparatus. Lysosomes are found in varying amounts in varying shapes but always consist of a central space filled with enzymes completely enclosed by a membrane.
- C** Lysosomes act as tiny vacuum cleaners, tidying up the cell and ingesting anything that there is too much of such as complex sugars and proteins. The substances they ingest are then broken down and released into the cell as smaller units for re-use. The breaking down is done by enzymes found only in the lysosomes. **The environment inside the lysosomes is more acidic than that of the surrounding cell cytoplasm, so only acidic enzymes can function inside the lysosome.**
- D** Enzymes are very sensitive to acidity and so enzymes which work inside the lysosome would not work outside and vice-versa. That is why Pompe disease does not have the same symptoms of low sugar levels that some of the other glycogen storage diseases produce – glycogen continues to be broken down normally in the cells outside the lysosomes and blood sugar levels are maintained. It is the excess glycogen that is taken into the lysosome that creates a problem.
- E** The reason that Pompe disease produces such severe symptoms is twofold. Firstly, **the missing enzyme is one of the lysosomal enzymes.** Pompe disease is referred to as a lysosomal storage disease. There are around 50 known lysosomal storage diseases. In each case there is a common problem: the lysosomes take up a substance that they do not possess the enzyme to break down. This means that **the lysosomes grow larger and larger until they disrupt cell function, and therefore, the function of the organ or tissue that the cells make up.**
- F** This leads us to the second reason that Pompe disease is so severe, even amongst lysosomal storage diseases. **As glycogen is the energy storage compound for the body, there is a lot of it in muscle.** This means that in Pompe disease, where there is no enzyme to break down the glycogen in the lysosomes, the lysosomes in the heart and skeletal muscle quickly accumulate large deposits of glycogen. As the lysosomes increase in size, the muscle cells are displaced. Additionally, when the lysosome bursts due to the large size, the acidic contents are released, degrading the surrounding tissue. The muscles are therefore progressively weakened.





# Looking Through A Father's Eyes

NAME

DATE

HOME GROUP MEMBERS

## A. Guiding Questions

Directions: Use the guiding questions 1-4 below as you share information about Pompe disease in your home group. Answer as thoroughly as possible. Include illustrations as needed.

1. What is an enzyme and how does it work?
  
  
  
  
  
  
  
  
  
  
  
  
  
  
  
2. Discuss the molecular basics from DNA → protein. What is a mutation and how does it affect the protein?
  
  
  
  
  
  
  
  
  
  
  
  
  
  
  
3. Is Pompe disease an inherited disorder? If so, describe how it is inherited and the probability.
  
  
  
  
  
  
  
  
  
  
  
  
  
  
  
4. What is a lysosome? What is its function?



LOOKING THROUGH A FATHER'S EYES (PAGE 2)

## B. Reflective Questions

Directions: Now that you have a shared understanding of the basic science of Pompe disease, answer the questions below 5-7 on your own.

5. What role does each of the above (enzyme, molecular basics, genetics, lysosome) play in Pompe disease?

6. Considering #5, what symptoms might you expect to see in an individual with Pompe disease?

7. How would you treat the disease? Think beyond simply "medicine" but what type of medicine? How would you administer it?



# Looking Through A Father's Eyes

1. What is an enzyme and how does it work?

An enzyme is a protein that catalyzes a reaction in a body. It has a specific confirmation that either holds molecules together or breaks them into their constituent parts.

2. Discuss the molecular basics from DNA → protein. What is a mutation and how does it affect the protein?

The four bases of DNA form three letter words. These words, called codons, code for specific amino acids. The amino acids form a chain which then folds into a functioning 3D structure. This structure is a protein. Some proteins are also enzymes. A mutation changes the DNA sequence, and therefore the resulting amino acid sequence. This alters the folding of the protein and can reduce or eliminate functionality of the protein.

3. Is Pompe disease an inherited disorder? If so, describe how it is inherited and the probability.

Pompe disease is a recessive disorder. Both parents must be carriers of the disease with a genotype of Aa. There is a 25% probability that heterozygous parents will have a homozygote recessive child, displaying the symptoms of Pompe disease.

4. What is a lysosome? What is its function?

Lysosomes are the vacuum cleaners of the cell. They take in unused molecules and debris and break it into constituent parts that can be discarded or reused by the cell. Lysosomes are acidic to aid in the breakdown of excess cellular materials.

5. What role does each of the above play in Pompe disease?

Pompe disease is an autosomal recessive disorder. Parents of an affected individual are both carriers of mutations in the GAA (acid alpha-glucosidase) enzyme and pass along the mutant copy to their child. The malfunctioning enzyme causes a build-up of glycogen in the lysosomes. As the lysosomes swell with the glycogen, muscle tissue is displaced. Additional damage is caused to the muscle cell when the lysosomes burst, releasing acidic contents to the surrounding cellular environment.

6. What symptoms might you expect to see in an individual with Pompe disease?

Muscle tissue is most affected since it contains a large amount of glycogen (needed for all of the energy expended by those cells). Therefore the symptoms would be related to large muscle groups. The severity of the disease form is going to be reflected in the muscles affected. (Extra information for the teacher: In infants, the heart is severely affected, leading to early death. The juvenile form is the next in severity involving respiratory muscles and large muscle groups. These individuals do not have as much cardiac involvement as the infant form, but many become ventilator and wheelchair dependent. With the late onset form, the major motor muscles are primarily involved.)

7. How would you treat the disease? Think beyond simply "medicine" but what type of medicine? How would you administer it?

Pompe is treated with enzyme replacement therapy (ERT). The protein (enzyme) must be directly administered to breakdown the glycogen in the lysosome. The difficult part is actually getting the enzyme into the lysosome. Just administering the enzyme through the bloodstream does not work; it accumulates in the liver. It needs to have a sugar residue added so that it will be absorbed into the acidic lysosome. The eventual hope is for a cure which would come in the form of gene therapy. With gene therapy, new DNA is introduced to the cells that have the functioning form of the GAA enzyme, and the normal cellular machinery would then be able to produce functioning GAA.



# Section Name Cards (page 1)

Copy and cut. Distribute one card per student. (Laminate for repeated use.)

Lysosome	Lysosome
Lysosome	Lysosome
Lysosome	Lysosome
Lysosome	Lysosome

Enzyme	Enzyme
Enzyme	Enzyme
Enzyme	Enzyme
Enzyme	Enzyme



# Section Name Cards (page 2)

Copy and cut. Distribute one card per student. (Laminate for repeated use.)

Molecular Basics

Molecular Basics

Molecular Basics

Molecular Basics

Molecular Basics

Molecular Basics

Molecular Basics

Molecular Basics

Genetics

Genetics

Genetics

Genetics

Genetics

Genetics

Genetics

Genetics



Notes:

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# 2

## The Road to Treatment

### Vocabulary:

**Enzyme replacement therapy:** a medical treatment replacing an enzyme in patients in whom that particular enzyme is deficient or absent. Usually this is done by giving

the patient an intravenous (IV) infusion containing the enzyme. Enzyme replacement therapy does not “treat” the underlying disease, only the symptoms.

### Pharmacological chaperone:

Small molecules that selectively bind to and stabilize proteins in cells, leading to improved protein folding and trafficking, and increased activity.

### ? KEY QUESTION(S):

- What is Pompe disease?
- What is the fundamental biology of the disease?

### 🕒 TIME ESTIMATE:

- Advanced Preparation: 30 minutes
- Student Procedure: 30 minutes

### 🎯 LEARNING STYLES:

- Visual, auditory, and kinesthetic.

### Lesson Summary:

Working in groups, students will read Pompe fact cards and use text clues to sequence the events in the discovery and treatment of Pompe disease. This lesson illustrates scientific discovery as a collaborative effort of many individuals building on prior knowledge and developing unique ideas to explore.

### Student Learning Objectives:

The student will be able to...

1. Sequence scientific discoveries
2. Discover that science is a collaborative effort
3. Consider the role technology has played in the rapid advances in biomedical science during the last twenty years

### Standards:

See table on page 11-13.

SC.912.L.16.10	SC.912.N.1.1	SC.912.N.1.3	SC.912.N.1.7	SC.912.N.2.5
SC.912.L.18.1	SC.912.N.1.2	SC.912.N.1.5	SC.912.N.2.4	

### Materials:

- *Student Page: The Road to Treatment Timeline Cards* (1 set per group)
- *Teacher Page: The Road to Treatment Timeline Cards for Wall* (1 set for teacher use)
- *Student worksheet: The Road to Treatment Timeline Cards* (1 per student or student group)

### Background Information:

Background information needed for this assignment is at the beginning of the guide and included on the information cards. Teachers should read the information cards prior to the start of the lesson.



### EXTENSION ACTIVITIES:

Have students investigate a stakeholder involved with the research and/or clinical aspects of Pompe disease.

Students may be curious about Genzyme's role and business motives behind acquiring all competing labs. This can lead to a great discussion about the business of major pharmaceutical companies. The topic will come up again in the final lesson of a gene therapy role play.

## Advance Preparation:

1. Prepare the student timeline cards for each student pair or group: Print one set single sided and write the corresponding letter on the back to spell POMPE DISEASE (see answer key and teacher timeline cards for order); copy and cut a set for each student pair or group. For extended use, consider cardstock and/or laminating.
2. Prepare the wall timeline cards. For extended use, consider cardstock and/or laminating.
3. Make copies of the student worksheet, one per student or student group.
4. Draw a timeline on the board or on the wall to affix the enlarged cards. Include 1930 – 2011.

## Procedure and Discussion Questions with Time Estimates:

1. **(5 min)** Use the flipchart to restate what the students learned yesterday (or call on student volunteers to share.) Probe to see if anyone did research on Pompe last night. If so, what did they learn?
2. **(2 min)** Tell the students they will now look at historical and current (2011) events and place them in chronological order. Have the students work in groups of 2-4. If the home groups from the previous day worked well together, you may wish to use the same groupings.
3. **(3 min)** Once students are assembled in groups of 2-4 and settled, distribute one envelope of time line cards to each group.
4. **(15 min)** Allow the students to order the cards and complete the worksheet. Move around the groups and alert them to clues in the cards if needed.
5. **(10 min)** Using the teacher timeline, place the first card on the timeline.
6. Call on a group to place the next as they give their one sentence summary. Ask the class if they agree with this choice.
7. Continue around the groups until all cards have been placed, addressing disagreements by asking questions to lead the students to the correct answer.
8. Once the timeline is complete, help lead students to the following conclusions:
  - a. The disease was characterized rather recently.
  - b. It has been a worldwide effort to understand and treat Pompe disease.
  - c. The advances in the last 15 years have been quite incredible. Ask the students why?

Awareness and technological advances have pushed the research. Biotechnology has provided the tools and techniques needed. Also, since Pompe is a rare disease, there are special incentives for research and clinical trials involving orphan drugs. Often, orphan drugs are pushed through faster since there is no alternative treatment, and lessons can be learned and applied to treatment for other less severe illnesses.

## Assessment Suggestions:

- Student worksheet can be collected. If student timeline cards are sequenced correctly, they spell POMPE DISEASE.

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### LITERATURE:

- Therapeutic approaches in Glycogen Storage Disease type II (GSDII)/Pompe disease [http://amdapompe.ehclients.com/downloads/publications/Therapeutic\\_Approaches.pdf](http://amdapompe.ehclients.com/downloads/publications/Therapeutic_Approaches.pdf)

### RESOURCES/REFERENCES:

- The Myozyme Story from Genzyme: <http://www.genzyme.com/pompemovie/>
- How is Lumizyme made? (has a very informative biomanufacturing video) [http://www.lumizyme.com/patients/about\\_lumizyme/how\\_lumizyme\\_is\\_produced.aspx](http://www.lumizyme.com/patients/about_lumizyme/how_lumizyme_is_produced.aspx)





# Timeline Activity Cards (page 1)

Copy and cut. Distribute one set per group. (Laminate for repeated use.)

**1 Dr. Joannes Cassianus Pompe** carried out a postmortem on a 7-month old girl who had died of pneumonia. He found the enlarged heart now known to be characteristic of the infantile form of the disease and had some microscope slides prepared. These showed that the muscle tissue was distorted into an oval mesh. Dr. Pompe later characterized the second glycogen storage disorder (GSD II) which now bears his namesake.

**2 G T Cori** described what are now known as glycogen storage disease types 1-4. Only in the case of Pompe disease (glycogen storage disease type 2) was the missing enzyme and its location unknown. The key to solving the puzzle of what caused Pompe disease lay in a seemingly unrelated discovery taking place elsewhere. Gerty and Carl Cori won a joint Nobel Prize for their work on glycogen metabolism.

**3 Christian de Duve** and co-workers were investigating the effect of insulin on the liver, when they discovered intra-cellular particles which seemed to have digestive properties. de Duve named these particles lysosomes. The idea that cells themselves had compartments (now known as organelles) with particular functions was now firmly established. Thus was the idea of the lysosome as the 'recycling plant' of the cell established. Prof de Duve was later awarded the Nobel Prize for his discoveries.

**4 Henri-Gery Hers** discovered a new enzyme, alpha-glucosidase, and knew it worked best at an acid pH. This set him thinking about his previous work with de Duve on lysosomes which had an acid environment. He further deduced that the normal function of this enzyme was to break down glycogen inside the lysosomes and that, in its absence, glycogen would accumulate. He established the concept of lysosomal storage diseases based on his Pompe disease research.

**5** There were attempts at a treatment for Pompe disease. Soon after Hers characterized lysosomal storage diseases, it had been suggested that enzyme replacement therapy might be a potential treatment. This was tried for Pompe disease, first using enzyme prepared from the fungus *Aspergillus niger* and later with enzyme derived from human placenta. All attempts failed; the enzyme was simply soaked up by the liver and did not reach the muscles.

**6 Arnold Reuser**, a researcher at Erasmus University in Rotterdam, and his then PhD student, **Ans van der Ploeg**, looked again at enzyme replacement therapy. In particular, they made use of the recent discovery that enzymes made their way into the lysosome using a receptor for the sugar mannose-6-phosphate. Their first experiment was to take cell lines isolated from Pompe patients – human muscle cells grown in a dish in the laboratory. Reuser & Van der Ploeg added the phosphorylated enzyme – and the glycogen was degraded. Reuser cloned the gene and shared with YT Chen.



# Timeline Activity Cards (page 2)

Copy and cut. Distribute one set per group. (Laminate for repeated use.)

**7** In 1996, the Rotterdam group joined with a biotech company called Pharming to produce the enzyme **alpha glucosidase (acid maltase)** in the milk of transgenic rabbits. Pharming got off to a quick start, announcing the start of Phase I clinical trials on April 15, 1998. This stage of clinical trials involves dosing healthy human volunteers to check of toxic effects. This was followed in July 1998 by the news that Pharming had entered into a partnership with Genzyme.

**8** Duke University announced that YT Chen's group would also be conducting clinical trials of ERT, in collaboration with a company called Synpac Pharmaceuticals. They would be using the more 'traditional' method of producing the GAA enzyme in Chinese hamster ovary (CHO) cells engineered to produce the human enzyme. This meant that there was now real competition, with a race to be first to announce results and to commercialize the product.

**9** Genzyme entered into agreements or acquired all parties working on ERT including Pharming, Synpac, and Novazyme. Additionally, they were working on their own internal enzyme. Genzyme initiated a major effort to study and compare the four drugs to determine which candidates offered the best chance of success in treating Pompe disease. This effort involved extensive research and analysis – the undertaking so large and so important it was nicknamed "The Mother of All Experiments" by the research team leaders. The winner would later be named Myozyme.

**10** After two global clinical research studies conducted at seven study sites, Myozyme was approved for the treatment of infant and juvenile forms of Pompe disease in the U.S. (April 28) and European Union (March 29) and it remains the only approved therapy for the treatment of Pompe disease in the world today. Additional clinical trials indicated ERT is effective in patients with late onset Pompe disease as well. Myozyme is repackaged as Lumizyme and approved for use in the US.

**11** In 2010 enrollment in the first gene therapy clinical trial was initiated. This phase I/II study is designed to target respiratory insufficiency which is the most life-threatening manifestation of Pompe. The vector is directly administered to the diaphragm. The target population for this study is children aged 3 – 14 who are dependent on mechanical ventilation despite ERT. These children represent the more severely affected spectrum of Pompe patients and the population most in need of improved therapeutic strategies.

**12** After previously halting clinical trials due to adverse effects, Amicus Therapeutics announced the initial infusion of the first subject in an open-label Phase 2 drug-drug interaction study of AT2220 (a pharmacological chaperone) co-administered with enzyme replacement therapy (ERT) in individuals with Pompe disease. The announcement was made in December, 2011.



# Timeline Wall Cards (page 1)

Copy and cut to display on wall. (Laminate for repeated use.)

## 1930

**P DR. JOANNES CASSIANUS POMPE** carried out a postmortem on a 7-month old girl who had died of pneumonia. He found the enlarged heart now known to be characteristic of the infantile form of the disease and had some microscope slides prepared. These showed that the muscle tissue was distorted into an oval mesh. Dr. Pompe later characterized the second glycogen storage disorder (GSD II) which now bears his namesake.



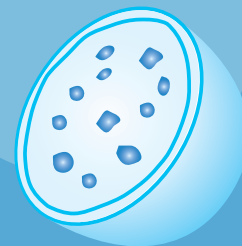
## 1947

**G GERTY CORI** described what are now known as glycogen storage disease types 1-4. Only in the case of Pompe disease was the missing enzyme unknown. The key to solving the puzzle of what caused Pompe disease lay in a seemingly unrelated discovery taking place elsewhere. Gerty and Carl Cori won a joint Nobel Prize for their work on glycogen metabolism.



## 1955

**M CHRISTIAN DE DUVE** and co-workers were investigating the effect of insulin on the liver, when they discovered intra-cellular particles which seemed to have digestive properties. de Duve named these particles lysosomes. The idea that cells themselves had compartments (now known as organelles) with particular functions was now firmly established. Thus the idea of the lysosome as the 'recycling plant' of the cell was established. Prof de Duve was later awarded the Nobel Prize for his discoveries.



**LYSOSOME**



# Timeline Wall Cards (page 2)

Copy and cut to display on wall. (Laminate for repeated use.)

1965

**P HENRI-GERY HERS** discovered a new enzyme, alpha-glucosidase, and knew it worked best at an acidic pH. This set him thinking about his previous work on lysosomes which had an acidic environment. He further deduced that the normal function of this enzyme was to break down glycogen inside the lysosomes and that, in its absence, glycogen would accumulate. He established the concept of lysosomal storage diseases based on his Pompe disease research.



1965-75

**E** There were attempts at a treatment for Pompe disease. Soon after Hers discovered lysosomal storage diseases, it had been suggested that enzyme replacement therapy might be a potential treatment. This was tried for Pompe disease, first using enzyme prepared from the fungus *Aspergillus niger* and later with enzyme derived from human placenta. All attempts failed; the enzyme was simply soaked up by the liver and did not reach the muscles.



Published  
1991

**D ARNOLD REUSER**, a researcher at Erasmus University in Rotterdam, and his then-PhD student, **ANS VAN DER PLOEG**, looked again at enzyme replacement therapy. In particular, they made use of the recent discovery that enzymes made their way into the lysosome using a receptor for the sugar mannose-6-phosphate. Their first experiment was to take cell lines isolated from Pompe patients – human muscle cells grown in a dish in the laboratory. Reuser & Van der Ploeg added the phosphorylated enzyme – and the glycogen was degraded. Reuser cloned the gene and shared with YT Chen.



# Timeline Wall Cards (page 3)

Copy and cut to display on wall. (Laminate for repeated use.)

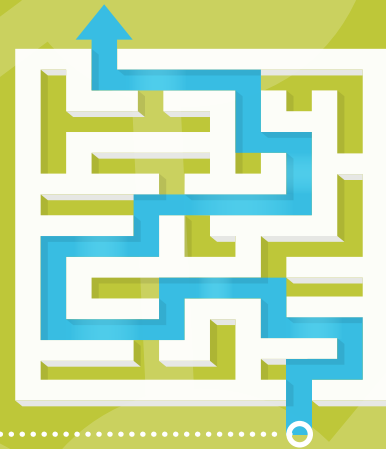
Published  
**2000**

**I** In 1996, the Rotterdam group joined with a biotech company called Pharming to produce the enzyme alpha glucosidase (acid maltase) in the milk of transgenic rabbits. Pharming got off to a quick start, announcing the start of Phase I clinical trials on April 15, 1998. This stage of clinical trials involves dosing healthy human volunteers to check of toxic effects. This was followed in July 1998 by the news that Pharming had entered into a partnership with Genzyme to produce the Pompe Enzyme Replacement Therapy (ERT).



Published  
**2001**

**S** Duke University announced that YT Chen's group would also be conducting clinical trials of Enzyme Replacement Therapy (ERT), in collaboration with a company called Synpac Pharmaceuticals. They would be using the more 'traditional' method of producing the GAA enzyme in Chinese hamster ovary (CHO) cells engineered to produce the human enzyme. This meant that there was now real competition, with a race to be first to announce results and to commercialize the product.



**2002**



**E** Genzyme entered into agreements or acquired all parties working on Enzyme Replacement Therapy (ERT) including Pharming, Synpac, and Novazyme. Additionally, they were working on their own internal enzyme. Genzyme initiated a major effort to study and compare the four drugs to determine which candidates offered the best chance of success in treating Pompe disease. This effort involved extensive research and analysis – the undertaking so large and so important it was nicknamed “The Mother of All Experiments” by the research team leaders. The winner would later be named Myozyme.

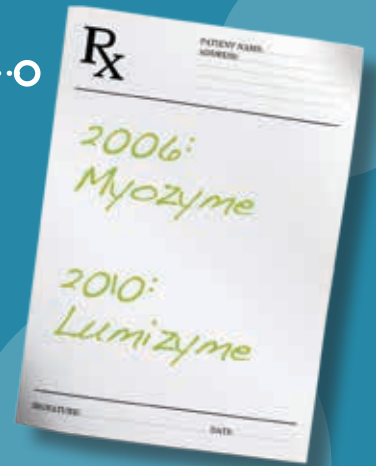


# Timeline Wall Cards (page 4)

Copy and cut to display on wall. (Laminate for repeated use.)

## 2006 & 2010

**A** After two global clinical research studies conducted at seven study sites, Myozyme was approved for the treatment of infant and juvenile forms of Pompe disease in the U.S. (April 28) and European Union (March 29) and it remains the only approved therapy for the treatment of Pompe disease in the world today. Additional clinical trials indicated ERT is effective in patients with late onset Pompe disease as well. Myozyme is repackaged as Lumizyme and approved for use in the US.



## 2010

**S** In 2010 enrollment in the first Pompe gene therapy clinical trial was initiated. This phase I/II study is designed to target respiratory insufficiency which is the most life-threatening manifestation of Pompe. The vector is directly administered to the diaphragm. The target population for this study is children aged 3 – 14 who are dependent on mechanical ventilation despite Enzyme Replacement Therapy (ERT). These children represent the more severely affected spectrum of Pompe patients and the population most in need of improved therapeutic strategies.



## 2011

**E** After previously halting clinical trials due to adverse effects, Amicus Therapeutics announced the initial infusion of the first subject in an open-label Phase 2 drug-drug interaction study of AT2220 (a pharmacological chaperone) co-administered with enzyme replacement therapy (ERT) in individuals with Pompe disease. The announcement was made in December, 2011.





# The Road to Treatment

NAME \_\_\_\_\_

DATE \_\_\_\_\_

Directions: Complete the chart below based on the timeline cards.

Date	ONE SENTENCE SUMMARY OF CARD	Letter on Card
1930		
1947		
1955		
1965		
1965-1975		
1991		
1996-2000		
2001		
2002		
2006-2010		
2010		
2011		



# The Road to Treatment

Directions: Complete the chart below based on the timeline cards.

Date	ONE SENTENCE SUMMARY OF CARD	Letter on Card
1930	Dr. Pompe describes pathology of then unknown disease.	P
1947	Dr. Gerty Cory and husband describe glycogen storage diseases.	O
1955	De Duve discovers lysosomes.	M
1965	Hers discovers the enzyme alpha glucosidase, its optimal function at an acidic pH, and describes lysosomal storage diseases.	P
1965-1975	Attempts to extract enzyme from fungus and placenta and use enzyme replacement therapy unsuccessful. Enzyme absorbed by liver and did not reach the muscles.	E
1991	Modified enzyme for uptake by lysosome in human cell line.	D
1996-2000	Published use of transgenic rabbits in clinical trials to produce needed enzyme.	I
2001	Use of CHO cells to produce GAA enzyme and begin clinical trials.	S
2002	Genzyme acquires all groups working on various ERT.	E
2006-2010	First commercial ERT product available. Myozyme in 2006 for infant and juvenile forms; repackaged as Lumizyme in 2010 for late onset form.	A
2010	Enrollment begins for the first Pompe gene therapy trial.	S
2011	Clinical trials for chemical chaperone resume.	E



# Pompe Journal Club

3

## Vocabulary:

**Peer-review** is the act of having another writer read what you have written and respond in terms of its effectiveness. This reader attempts to identify the

writing's strengths and weaknesses, particularly how sound the science is, and then suggests strategies for revising it. The hope is that not only will the specific piece of writ-

ing be improved, but that future writing attempts will also be more successful. Peer-review happens with all types of writing, at any stage of the process, and with all levels of writers.

## Lesson Summary:

Using a reading guide, students work in pairs to read a journal article and share their interpretations during a whole class activity. After completing the introductory reading activity, students utilize a reading guide to work independently to read and identify key information before sharing their understanding with others in a small group. If time allows, students translate their paper into a poster to share with classmates during a gallery walk and subsequent poster presentation.

## Student Learning Objectives:

The student will be able to...

1. Identify key developments in the treatment of Pompe disease
2. Improve scientific literacy by reading primary sources
3. Read a scientific paper for understanding
4. Recognize that science is ever growing and building on previous discoveries
5. Conclude that research takes place around the globe and through publishing, discoveries are shared

## Standards:

See table on page 11-13.

SC.912.L.14.6	SC.912.L.18.1	SC.912.N.1.3	SC.912.N.1.5	SC.912.N.2.5
SC.912.L.16.9	SC.912.N.1.2	SC.912.N.1.4	SC.912.N.2.4	

## Materials:

- Copies of *Guide to Reading Scientific Papers*, one per student
- Copies of *Guide to Reading Scientific Papers Worksheet*, one per student
- Copies of introductory journal article, one per student or student pair
- Copies of journal articles (choose three to five articles for the class and make multiple copies of each, allowing one per student)

## KEY QUESTION(S):

- How do you read a scientific journal article for understanding?

## TIME ESTIMATE:

- Advanced Preparation: 90 minutes including reading articles
- Student Procedure:  
Day 1: 45 minutes  
Day 2: 45 minutes  
Day 3: 30 minutes

## LEARNING STYLES:

- Visual and auditory



Small, manageable doses of primary literature in a guided manner will allow students to gain comfort using and understanding original research articles.

## Background Information:

Little was known about Pompe disease until the latter part of the 20<sup>th</sup> century. There have been several reviews of literature and reported cases published, but most attention from the research and clinical community is focused on treatment and cure. Therefore many of the papers published are related to a treatment and heavy on academic jargon.

Students need to be scientifically literate, and part of that effort includes their use and familiarity with primary sources of information, namely articles peer-reviewed and published in scientific journals. Many high school and undergraduate students are intimidated by these articles because they approach them in the wrong manner. They are not novels to be consumed at one time. They are jargon-filled texts that are often presented in a dry and painful manner. Students are bogged down by the vocabulary and the methods, so they can not focus on the bigger, important ideas of the paper, the “take-home message”. Small, manageable doses of primary literature in a guided manner will allow students to gain comfort using and understanding original research articles.

## Advance Preparation:

**Implementation note:** Determine size of groups for journal discussions and choose papers accordingly. For a class of 32 students, if the journal clubs (groups) are 8 students each you will need to select 4 papers and make 8 copies of each. The usual standard is to keep groups as small as possible. In this situation however, a group of 6-8 students discussing a paper would be fine and could encourage discussion and generate more ideas. If you opt for smaller groups of 4, you will need to select (and read!) 8 papers.

- Make copies of *Guide to Reading Scientific Papers*, one per student
- Select and copy journal articles (choose three to five articles for the class, depending on how large the groups will be, and make multiple copies of each, allowing one per student). Most research journals have restricted access. The AMDA has a site with many important publications related to Pompe disease and it is available freely. (<http://www.amda-pompe.org/index.php/main/research/publications/>) Suggested articles are listed in the resources below.
- Read the selected articles.

### Implementation Notes:

This activity is intended to build on the discoveries presented in the previous timeline lesson. It also provides background information for the gene therapy role play and subsequent ethics class discussion later in the unit. As written, three class days are devoted to this activity, stressing the importance of communicating science. Students need to know where to find primary sources of scientific literature as well as how to read and present the information for understanding. Due to the rare nature of Pompe disease, there has not been much written in popular media outlets other than a few first person accounts and many of those are the result of publicity surrounding the movie *Extraordinary Measures*.

It also assumes students are not familiar with reading science journal articles so it models the steps of reading and discussing a paper as the first part of the lesson. For advanced and/or experienced students, you may elect to skip the day one component and begin with assigning students papers to read for homework and discuss in small groups the following day.

For classes accustomed to the use of blogs or other social media outlets, this lesson would be well suited for discussion to take place outside of the classroom. In this situation, you may wish to assign the entire class a paper one night and ask them to respond to a blog post or wiki prompt. They could repeat this process for multiple papers throughout the unit. The poster and gallery walk could also be held virtually through the use of glogs.

## Procedure and Discussion Questions with Time Estimates:

### DAY ONE (45 MIN)

- (5 min)** Introduce the importance of publishing scientific findings. Tell the students that in science, information is primarily shared with others through writing papers that are reviewed by other scientists for accuracy and clarity (peer-review). These publications are the primary means to share new findings with others across the world and allow researchers to build on prior findings to make new discoveries. It is important for scientists to be good written and oral communicators.
- (5 min)** Give each student a copy of *Guide to Reading Scientific Papers*. Instruct the students to read the *Guide* silently.
- While students are reading, provide a copy of the first article to each student or student pair.
- Project the first article (feel free to select a different article) Disease severity in children and adults with Pompe disease related to age and disease duration [http://amdapompe.ehclients.com/downloads/publications/Hagemans\\_Neurology\\_280605.pdf](http://amdapompe.ehclients.com/downloads/publications/Hagemans_Neurology_280605.pdf)
- (5 min)** Review the *Guide to Reading Scientific Papers* with them, stressing which sections students should read a bit more carefully (abstract, introduction), and which can be skimmed or skipped (methods/materials). Use the projected article to indicate these sections. Direct their attention to the questions at the bottom to focus their reading. You may wish to have students highlight or underline the answers on the article.
- (15 min)** Ask the student pairs to work together to read the article and formulate a summary. Encourage them to use the *Guide to Reading Scientific Papers*. Point out that to read a paper for full comprehension, it takes longer, but this is a beginning exercise to get them acquainted with scientific papers.
- (10 min)** Call on student pairs to give a summary of each key section (abstract, introduction, results, and conclusions). The *Teachers Page: Discussing Science Journals* provides a teacher's guide for this discussion. Ask other students to add to the summary that has already been given, asking them if they thought there were other important aspects. Prompt them with the questions below:
  - What was the purpose of the study?
  - What questions were asked?
  - What were the final answers?
  - What was unique about the study?
  - What is the next step?
- (5 min)** Distribute a copy of one paper to each student. Choose 3-5 papers so there will be multiple students reading the same paper.
- Instruct the students to follow the same procedure they just did as a class tonight for homework, using the *Guide to Reading Scientific Papers* and completing the *Guide to Reading Scientific Papers Worksheet*. Tell them to be prepared to share their paper in small groups tomorrow.

### DAY TWO (45 MIN)

- Present students with the following quick write prompt as a bell ringer:
  - In three complete sentences, summarize the journal article you read last night.
  - List one thing you learned.
  - List one question you still have.
- Allow students 5 minutes to complete the prompt. Collect the writings.



In science, information is primarily shared with others through writing papers that are reviewed by other scientists for accuracy and clarity (**peer-review**). These publications are the primary means to share new findings with others across the world and allow researchers to build on prior findings to make new discoveries. It is important for scientists to be good written and oral communicators.

3. **(15 min)** Ask students to gather in groups according to the paper they read. Allow the students to self-sort. Instruct them that within the group, they should share their understanding of the paper, discuss anything they did not understand, and decide on the important points using the questions in the *Guide* as prompts. This is similar to a journal club in a research setting.
4. **(10 min)** After the students have had the opportunity to discuss their paper, they will now make a poster using the five questions from their reading guide to share with the class during a gallery walk.

**Implementation note:** *Instead of posters and a gallery walk, student could do a modified jigsaw, forming groups with members from different papers. Each should share with the new group the summary and important points from their individual paper. Allow 20 minutes for the groups to assemble and share. Have them complete an exit slip during the last 5 minutes asking them to state one thing they learned and one thing they still have a question about.*

5. **(15 min)** Have groups affix their poster to the wall when completed. Students should move around the room and read the other posters. Give them post-it notes and ask them to generate at least one question and place it next to the corresponding poster (similar to an exit slip). It cannot be the same question someone else has already posted. (If wanting to use for assessment purposes, students should include their name on the back of the post-it.)
6. Teacher homework: review the quick writes and make note of any reoccurring questions or concerns expressed by the students. Review the post-it note exit slips and make note of thoughtful questions to address the following day. As the student groups are presenting to the class, prompt them with the questions and provide wrap-up for each poster after the students are finished to clarify any misunderstandings.

### DAY THREE (45 MIN)

1. As students enter, they should visit their poster and read the questions their classmates posted.
2. **(5 min)** Encourage student groups to discuss the questions posed and decide on a main speaker for the group to present the paper abstract; the additional members of the group should each answer one of the questions posed by their classmates.
3. **(5-10 mins)** Allow the first group 3-5 minutes to present their paper/poster to the class and answer questions. (The number of questions answered may vary based on complexity of the answer and classroom engagement.) Help to clarify concepts and correct any misconceptions. The questions might be beyond the scope of knowledge for the students, so it is critical that the teacher is ready to assist with answering, correcting, or encouraging students to investigate further to find the answer.
4. **(20 min)** Repeat with the remaining groups.
5. **(10 min)** When all groups have presented, engage in a whole class discussion about the findings of the papers, focusing the students' attention on the following:
  - a. Did it build on previous work?
  - b. Where does it fit in our timeline from lesson 2?
  - c. Key finding(s)
6. **(5 min)** Help the students understand that science is an ever-growing body of knowledge. Only by sharing results can forward progress be made.

### Assessment Suggestions:

- Homework and/or quickwrite collected
- Participation grades for the exit slip and paper/poster presentation.

## RESOURCES/REFERENCES:

How to read scientific papers:

[http://hampshire.edu/~apmNS/design/RESOURCES/HOW\\_READ.html](http://hampshire.edu/~apmNS/design/RESOURCES/HOW_READ.html)

Nice list of research tools including how to read a research article and citations.

[https://pantherfile.uwm.edu/ajpetto/www/Research\\_tools.htm](https://pantherfile.uwm.edu/ajpetto/www/Research_tools.htm)

The AMDA has an extensive list of publications with linked pdfs. The suggested articles below can be found on their site:

<http://www.amda-pompe.org/index.php/main/research/publications/>. There are

additional articles in the References section at the end of this curriculum (as well as full citations for the links below) that would be excellent for students to read and discuss.

Unfortunately, access is limited. For assistance accessing a desired article, feel free to contact us at [julie@cpet.ufl.edu](mailto:julie@cpet.ufl.edu).

### Disease Pathology

Disease severity in children and adults with Pompe disease related to age and disease duration

[http://amdapompe.ehclients.com/downloads/publications/Hagemans\\_Neurology\\_280605.pdf](http://amdapompe.ehclients.com/downloads/publications/Hagemans_Neurology_280605.pdf)

### Enzyme Replacement Therapy

Autophagy and Mistargeting of Therapeutic Enzyme in Skeletal Muscle in Pompe Disease

<http://amdapompe.ehclients.com/downloads/publications/autophagy.pdf>

Clinical and Metabolic Correction of Pompe Disease by Enzyme Therapy in Acid Maltase-deficient Quail

<http://amdapompe.ehclients.com/downloads/publications/JCI1014827.pdf>

Long-Term Intravenous Treatment of Pompe Disease With Recombinant Human -Glucosidase From Milk.

<http://amdapompe.ehclients.com/downloads/publications/rabbitGAA.pdf>

A Randomized Study of Alglucosidase Alfa in Late-Onset Pompe's Disease

<http://amdapompe.ehclients.com/downloads/publications/nejmoa0909859.pdf>

### Myozyme Clinical Trial

Early Treatment With Alglucosidase Alfa Prolongs Long-Term Survival of Infants With Pompe Disease

[http://amdapompe.ehclients.com/downloads/publications/Early\\_Treatment\\_With\\_Alglucosidase\\_Alfa\\_Prolongs16.pdf](http://amdapompe.ehclients.com/downloads/publications/Early_Treatment_With_Alglucosidase_Alfa_Prolongs16.pdf)

Effect of enzyme therapy in juvenile patients with Pompe disease: A three-year open-label study.

[http://amdapompe.ehclients.com/downloads/publications/MiniLOTS\\_Genzyme\\_Sponsored\\_Single\\_Center\\_Study.pdf](http://amdapompe.ehclients.com/downloads/publications/MiniLOTS_Genzyme_Sponsored_Single_Center_Study.pdf)

### Gene Therapy

Pompe disease gene therapy.

<http://amdapompe.ehclients.com/downloads/publications/Byrne-HMG2011.pdf>

A New Method for Recombinant Adeno-associated Virus Vector Delivery to Murine Diaphragm

<http://amdapompe.ehclients.com/downloads/publications/VirusDeliveryDIAPHRAGM.pdf>

Efficacy of an Adeno-associated Virus 8-Pseudotyped Vector in Glycogen Storage Disease Type II

<http://amdapompe.ehclients.com/downloads/publications/Amalfitano2005.pdf>

Spinal Delivery of AAV Vector Restores Enzyme Activity and Increases Ventilation in Pompe Mice

<http://amdapompe.ehclients.com/downloads/publications/byrne.pdf>

### Chemical Chaperones

Chemical chaperones improve transport and enhance stability of mutant a-glucosidases in glycogen storage disease type II

<http://amdapompe.ehclients.com/downloads/publications/chaperones.pdf>



## EXTENSIONS:

Students compose an essay or poster to inform the general public of Pompe disease or another orphan disease.

After students have completed the entire activity, have them read the following editorial: [http://amdapompe.ehclients.com/downloads/publications/Where\\_do\\_we\\_stand--Ans\\_letter.pdf](http://amdapompe.ehclients.com/downloads/publications/Where_do_we_stand--Ans_letter.pdf)

Based on what they have learned so far, do they agree with the author? Is there bias in her comments? Is it acceptable for the author to present her opinion in an editorial piece?



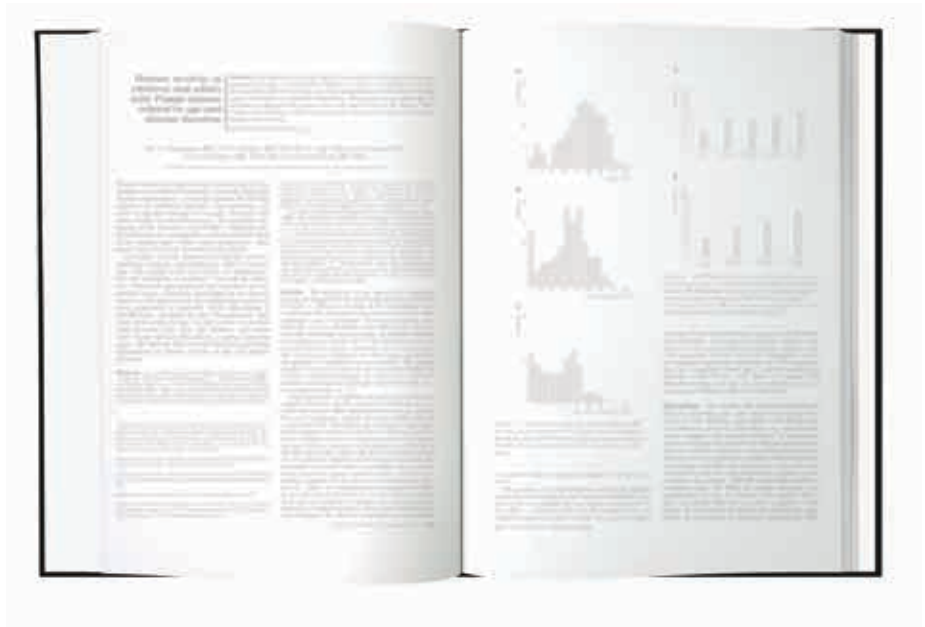
# Discussing a Science Journal Article

Disease severity in children and adults with Pompe disease related to age and disease duration [http://amdapompe.ehclients.com/downloads/publications/Hagemans\\_Neurology\\_280605.pdf](http://amdapompe.ehclients.com/downloads/publications/Hagemans_Neurology_280605.pdf)

Feel free to use any article. This one was selected to model because it is short and not very complicated, which will allow students to focus on the main parts and how to read an article rather than be burdened with jargon and methods during the introduction.

Things to point out:

1. Read the title: Disease severity in children and adults with Pompe disease related to age and disease duration.
2. Note the journal: *Neurology*. This is a peer-reviewed and highly respected journal.
3. Multiple authors contributed, indicating collaboration among many individuals. In this case, the funding for the project and the main research lab is indicated by the last name listed, Van der Ploeg.
4. In the bottom left, note the institutions represented, who funded the project, and potential conflict of interest. This information is telling regarding any bias that might be apparent or inadvertent.
5. Also, note the date the paper was originally received: Dec. 21, 2004. This is the date it was submitted to the journal. After going through peer review,



Each journal has its own way of arranging the text on the page, as well as specific sections they do or do not want included. Use the reading guide with the students, calling attention to each section and highlighting key points.

suggested modifications are sent to the authors for the chance to revise. The revision is then either approved or denied publishing. In this case, it was accepted on March 23, 2005 and appeared in print in June, 2005.

6. The layout of papers differ. Each journal has its own way of arranging the text on the page, as well as specific sections they do or do not want included.

Use the reading guide with the students, calling attention to each section and highlighting key points.

The **Abstract** provides a nice summary of the paper.

- This one is particularly short, reflective of a short paper. 255 individuals with Pompe completed a survey to gather information about the natural course of the disease.

The **Introduction** gives a history of the topic and discusses what



others have found. It also poses the research question(s).

- The author devotes the first paragraph to a description of Pompe. This provides background. They then discuss what the current treatment available is – ERT is just in clinical trials (this is why it is important to note the date). The authors want to help determine the ideal time to administer the treatment, so they need to understand the natural course of the disease, particularly for individuals with late-onset.

**Methods and Materials** are most meaningful to those in the field who might want to repeat the research or to help clarify results. Skip this section, but note that as you become more experienced with reading primary sources, it can be helpful to return to this section to better understand some of the results and discussion.

- Interesting how tiny the font is for the methods section in this journal, indicating this section is really for those that need to know all the details of carrying out the experiment. Not necessary for our students to understand the paper.

**Results** are just that. There is no discussion or explanation. They are worth a glance, particularly if any tables are included that summarize the findings neatly. Just a skim of this section will suffice.

- Font size is increased a bit, but not as large as the introduction or conclusions. There are a lot of percentages given, and description of the figures. It does give some nice

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**Results are just that. There is no discussion or explanation. They are worth a glance, particularly if any tables are included that summarize the findings neatly.**

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descriptions of the symptoms and pathologies affected individuals reported, and the age of onset.

The **Discussion/Conclusion** is where the author explains what happened. In this section, the questions should be answered. This is usually where the author reflects on the work and its meaning in relation to other findings and to the field in general.

- General findings are discussed such as disease severity increases with duration. This is a progressive disease, so not unexpected. It was not correlated with age however, supporting the diverse set of symptoms that present at all ages. Other than the classic infantile form, all other forms are heterogeneous. They do point out the subset of children who were affected very young, as presenting more severe symptoms earlier and consistently: those who are respirator dependent, are likely to be wheelchair dependent. Their concluding remarks suggest ERT should be started as early as possible, before “irreversible damage has occurred” such as muscle weakness requiring a wheelchair or respiratory assistance.

Add your own interpretations to these:

- What was the purpose of the study? Compile and analyze data about the natural course of Pompe disease (how does it progress in individuals not on enzyme replacement therapy?)
- What questions were asked? Is there a relation between age of onset, duration, and symptom severity?
- What were the final answers? Only in those presenting symptoms very young is there an expected course; all others present a mix of symptoms.
- What was unique about the study? Surveyed 255 patients, quite a large sample size for a disease so rare, to record the natural history of the disease with one standard questionnaire. Rather than piecing bits of case reports together for the literature, they were able to standardize the questions and therefore the results.
- What is the next step? Compare to the next generation who receives ERT. Does ERT make a difference in severity and duration? Can those who present symptoms early, be treated with enzyme replacement therapy and delay serious symptoms? For how long?



## Guide to Reading Scientific Papers

**S**cientific papers can be daunting, full of details and language that is unfamiliar. Scientific papers are best read and considered in small, manageable pieces. Unless you plan to repeat the experiment, you really just need to get the general idea of the questions and answers along with the big idea of the paper. As you become more comfortable with reading journal articles, you will naturally read for more depth and content. When starting out however, the key is knowing what to read, what to skim, and what to skip. Yes. There are parts of a paper that you can skip.

The paper is divided into sections, based generally on the scientific method. Most research papers contain the following sections: Abstract, Introduction, Methods/Materials, Results, Discussion, sometimes Conclusions, and References.

The **Abstract** provides a nice summary of the paper. It might have some unknown words or numbers, but it gives the overall flavor of the paper. It should be read and then re-read at the end.

The **Introduction** gives a history of the topic and discusses what others have found. It also poses the research question(s).

**Methods and Materials** are most meaningful to those in the field who might want to repeat the research or to help clarify results. Skip this section, but note that as you become more experienced with reading primary sources, it can be helpful to return to this section to better understand some of the results and discussion.

**Results** are just that. There is no discussion or explanation. They are worth a glance, particularly if any tables are included that summarize the findings neatly. Just a skim of this section will suffice.

The **Discussion/Conclusion** is where the author explains what happened. In this section, the questions should be answered. This is usually where the author reflects on the work and its meaning in relation to other findings and to the field in general.

Re-read the Abstract. Does it make more sense now? It should tie everything together.

Vocabulary. You may need to look words up if you can't figure them out using context clues. You can miss a really important point of the paper if you do not understand the language.

In summary:

- Absolutely read the Abstract, Introduction, Discussion, and then the Abstract again.
- Skim the results.
- Skip the methods/materials.

In the end, you want to be able to answer the following questions with some confidence:

- What was the purpose of the study?
- What questions were asked?
- What were the final answers?
- What was unique about the study?
- What is the next step?





# Guide to Reading Scientific Papers

NAME \_\_\_\_\_

DATE \_\_\_\_\_

1. What was the purpose of the study?

2. What questions were asked?

3. What were the final answers?

4. What was unique about the study?

5. What is the next step?



# Science Take-Out: From DNA to Protein Structure and Function

## 4

### Vocabulary:

**Transcription:** DNA → RNA; During transcription, a DNA sequence is read by an RNA polymerase, which produces a complementary, antiparallel RNA strand. The RNA complement includes uracil (U) in all instances where thymine (T) would have occurred in a DNA complement.

**Translation:** RNA → Protein; In translation, messenger RNA (mRNA) produced by transcription is decoded by the ribosome to produce a specific amino acid chain, or

polypeptide, that will later fold into an active protein.

**RNA:** Ribonucleic acid is one of the three major macromolecules (along with DNA and proteins) that are essential for all known forms of life. Like DNA, RNA is made up of a long chain of components called nucleotides. Each nucleotide consists of a nucleobase, a ribose sugar, and a phosphate group. RNA directs the synthesis of proteins.

**Genotype:** The genotype is the genetic makeup of

a cell, an organism, or an individual (i.e. the specific allele makeup of the individual). The genotype of an organism is the inherited instructions it carries within its genetic code.

**Phenotype:** A phenotype is the composite of an organism's observable characteristics or traits. Phenotypes result from the expression of an organism's genes as well as the influence of environmental factors and the interactions between the two.

### ? KEY QUESTION(S):

- How does a mutation at the DNA level affect the protein structure and function?

### 🕒 TIME ESTIMATE:

- Advanced Preparation: 30 minutes
- Student Procedure: 45 minutes

### 🎯 LEARNING STYLES:

- Visual and kinesthetic.

## Lesson Summary:

With a commercially available kit from Science Take-Out, students step through the process of transcribing and translating a DNA sequence. Using the student worksheet provided here, students then consider how the acid phosphatase gene is affected by mutations and how the change in structure affects the function of the enzyme.

## Student Learning Objectives:

The student will be able to...

1. Discuss observed inheritance patterns caused by recessive mode of inheritance
2. Describe the basic process of DNA replication and the transmission of genetic information
3. Explain how mutations in the GAA gene may or may not result in phenotypic change
4. Model the basic processes of transcription and translation and how they result in the expression of the GAA gene to produce a protein
5. Explain how and why the genetic code is universal and is common to almost all organisms



Genetic diseases are caused by negative mutations and can be passed along from one generation to the next. Pompe is an example of a genetic disease.

6. Describe the basic molecular structures and primary functions of DNA and proteins (biological macromolecules)
7. Construct proteins and amino acids structures. Explain the functions of proteins in living organisms. Relate the structure and function of enzymes.
8. Explain the role of enzymes.
9. Describe the function of models in science.

## Standards:

See table on page 11-13.

SC.912.L.14.6	SC.912.L.16.4	SC.912.L.18.1	SC.912.N.1.6
SC.912.L.16.2	SC.912.L.16.5	SC.912.L.18.4	SC.912.N.3.5
SC.912.L.16.3	SC.912.L.16.9	SC.912.L.18.11	

## Materials:

- Science Take-Out: *From DNA to Protein Structure and Function*, 1 kit per student group (recommend pairs)

Description	Source	Catalog #	Price
From DNA to Protein Structure and Function	Science Take-Out <a href="http://www.sciencetakeout.com">www.sciencetakeout.com</a>	STO-106 (individual assembled kit)	1 assembled kit: \$8
		STO-106U (unassembled classroom sets)	1 pk of 10 unassembled kits: \$38 <i>(bulk discounts available)</i>

## Background Information:

DNA is sometimes referred to as the blueprint of life. It holds the letters that code for almost all life on earth. Because DNA is so important, it has several proofreading and editing mechanisms in place to avoid errors as it replicates itself millions of times. However, occasionally there are mistakes. Those mistakes or mutations, changes in the DNA sequence, can lead to very different outcomes. Some are neutral. They occur in a region that does not affect the subsequent protein or the base change does not alter the amino acid codon. Some are beneficial. They cause a new protein to be made or alter the regulation of an existing protein, perhaps enabling the organism to gain an advantage in their environment. The ones we usually hear about however, are those that have a negative effect on the organism. Genetic diseases are caused by negative mutations and can be passed along from one generation to the next. Pompe is an example of a genetic disease.

In the case of Pompe disease, the acid alpha-glucosidase gene is damaged resulting in a protein that does not function correctly. There have been over 300 mutations identified to date, some with more devastating effects than others. A great deal of research is taking place to catalog the mutations and track potential correlations between genotype and phenotype. Some mutations are obviously deleterious, such as null mutations which prevent the amino

acids from being translated therefore no functioning protein is made. These mutations are predictive of early onset and the most severe form of Pompe disease.

In this lesson, students will practice the process of transcribing a sequence of DNA. Using complementary base pairing rules, substituting the RNA version of thymine, uracil, they will create the new single stranded RNA sequence. Since this RNA actually codes for a protein, it is mRNA and referred to as a transcript. Using the universal genetic code, three letter mRNA codons are translated into an amino acid chain. Amino acids have different chemical properties; they are hydrophobic or hydrophilic, acidic or basic. As the amino acid chain is folded into secondary, tertiary, and then a quaternary structure, the chemical properties of each amino acid affects the confirmation. Correct folding is crucial for enzymatic function.

**Implementation Note:** As written, the Science Take-Out Kit uses sickle cell disease and mutant hemoglobin as the protein example in part C to demonstrate a change in DNA can result in a change in protein structure and therefore function. This part has been modified in this curriculum to fit Pompe disease and GAA protein.

### Advance Preparation:

- Use preparation guide in the Science Take-Out kit.
- Copy student pages from Science Take-Out for each student, substituting Section C with the pages below.

### Procedure:

- Have students work in pairs.
- Follow procedure for part A of Science Take-Out kit. Optional: Part B can be completed for homework or as time allows in class. Substitute Part C below, which is adapted for Pompe.

### Assessment Suggestions:

- Student worksheet can be used for assessment.

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### RESOURCES/REFERENCES:

Science Take-Out: *From DNA to Protein Structure and Function* Catalog# STO-106 (individual assembled kit) and STO-106U (unassembled classroom sets)

Transcription video: <http://www.youtube.com/watch?v=WsofH466lqk>

Transcription video: <http://www.youtube.com/watch?v=5MfSYnltYvg&feature=related> (DNA Learning Center) also at <http://www.dnalc.org/resources/3d/index.html>

Translation video: <http://www.youtube.com/watch?v=8dsTvBaUMvw&feature=relmfu> (DNA Learning Center) also at <http://www.dnalc.org/resources/3d/index.html>

Transcription/translation video from PBS: [http://youtu.be/41\\_Ne5mS2ls](http://youtu.be/41_Ne5mS2ls)



### EXTENSIONS:

#### Activities

MSOE lending library – <http://cbm.msoe.edu/> has an amino acid starter kit that allows students to look closer at each amino acid <http://cbm.msoe.edu/teachRes/library/ak.html>

Transcription/translation 3D animations from DNA Learning Center: <http://www.dnalc.org/search?q=transcription>

Transcription/translation interactive animation from Utah (Learn Genetics) <http://learn.genetics.utah.edu/content/begin/dna/transcribe/> and

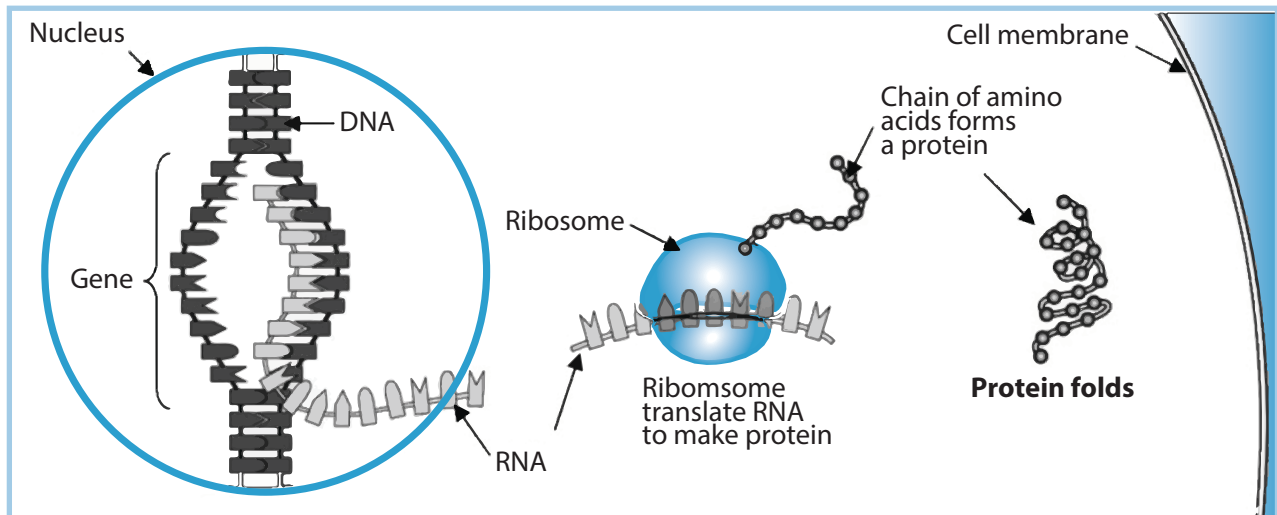
DNA to Protein: <http://learn.genetics.utah.edu/content/begin/dna/>

#### Literature

The National Institutes of Health *Structures of Life*, available in html, pdf, or print provides good background information about the role and importance of proteins. Chapter One is particularly relevant to this lesson. <http://publications.nigms.nih.gov/structlife/index.html>. May want to consider giving as reading homework prior to starting Lesson Four or as a review after the assignment.



# Science Take-Out: From DNA to Protein Structure and Function



The nucleus of every cell contains chromosomes. These chromosomes are made of DNA molecules. Each DNA molecule consists of many genes. Each gene carries coded information for how to make one type of protein.

Your body makes many different kinds of proteins. Every protein in your body has a specific shape. It is this specific shape that allows each protein to perform a specific job. The combination of specific proteins that your body makes gives you your traits.

During protein synthesis, DNA is copied in the nucleus to make RNA. The RNA then moves to the cytoplasm where it attaches to a ribosome. The ribosome translates the coded information from the RNA to create a protein molecule with a specific sequence of amino acids. The protein folds into a particular shape that enables it to perform a specific function.

To help you understand how proteins are produced and how they function, you will model the processes of protein synthesis and protein folding.

## YOUR TASKS:

- Model the processes of protein synthesis and protein folding.
- Relate the three dimensional (3-D) shape of proteins to their functions.

## A. Modeling Protein Synthesis and Protein Folding

1. Use the **From DNA to Protein – Record Sheet** in your lab kit. The illustration in Step A of this worksheet represents a small part of the DNA code in a gene that carries instructions for making one kind of protein.
2. DNA molecules and genes cannot leave the nucleus. To carry the instructions to the ribosomes (protein factories) in the cytoplasm, the coded information in a gene (DNA) base sequence is transcribed (copied) to make messenger RNA (mRNA) molecules with complementary (opposite) base sequences.
3. Use the base pairing chart below to determine the base sequence code on the mRNA that would be produced when the DNA molecule is transcribed to make mRNA. The first few mRNA bases have been provided as samples. Write your answer on the mRNA line on the diagram (**Step B of your worksheet**).

Base on DNA	Complementary Base on mRNA
A	U
T	A
G	C
C	G



## Science Take-Out: From DNA to Protein Structure and Function (page 2)

4. The mRNA then leaves the nucleus and attaches to a ribosome in the cytoplasm. In the ribosome, the mRNA base sequence is translated to determine the sequence (order) of amino acid building blocks in a protein.
5. Use the mRNA base sequence on your diagram and the genetic code chart provided to translate the mRNA code into a sequence of amino acid building blocks in a protein (also called a polypeptide). Each amino acid will be represented by a circle. The colors for the amino acids (yellow, blue, red or white) are indicated on the genetic code chart.
  - Write the name of the amino acids in the protein in the box above the circle (Step C of your Record Sheet).
  - Color each circle with the appropriate color as shown in the example. If you do not have colored pencils, you may write the color in the space below each of the circles.
6. Proteins are made of amino acids hooked end-to-end like beads on a necklace. To simulate a protein, you will make a model of your protein using a long chenille stem (the fuzzy covered wire) and colored beads. Refer to the sequence of 15 amino acids (the colored circles on Step C of your Record Sheet) and arrange beads of the appropriate colors on the chenille stem. The beads should be approximately evenly spaced along the chenille stem.
7. The model that you have created represents the unfolded, primary structure of a protein –which is the sequence of amino acids in a protein.
8. Proteins do not remain straight and orderly. They twist and buckle, folding in upon themselves to form 3-dimensional (3-D) shapes. Twist one half of your protein model around a pencil to make a spiral. This spiral region is called an “alpha-helix.”
9. Bend the other half of your protein molecule into a zig-zag shape by making a bend in the opposite direction at each bead. This zig-zag region is called a “beta-pleated sheet.”
10. The alpha-helix and beta-pleated sheet represent the secondary structure of a protein. Make a drawing to show the secondary structure of your protein model (Step D of your Record Sheet). Label the alpha helix region and the beta-pleated sheet region on your drawing.
11. Twist your protein into a 3-D shape according to these protein folding rules:
12. The resulting twisted structure is called the tertiary structure of a protein. Make a drawing to show the tertiary structure of your protein model (Step D of your Record Sheet). Note: Do the best you can to make your 2-dimensional drawing look like your 3D model.
13. Some protein chains are attracted to other protein chains. Work with a partner group and try putting your protein model next to their protein model in a way that still follows the rules of protein folding. This represents the quaternary structure of a protein.
14. Make a drawing to show the quaternary structure of your protein model (Step D of your Record Sheet).

Amino Acid (Bead) Color (based on chemical properties)	Protein Folding Rules
Yellow	These are fatty <u>hydrophobic</u> (water fearing) amino acids that should be clustered on the inside of the protein.
Blue	These are <u>negatively charged</u> basic amino acids that are attracted to red (positively charged) amino acids on the protein.
Red	These are <u>positively charged</u> acidic amino acids that are attracted to blue (negatively charged) amino acids on the protein.
White	These are <u>hydrophilic</u> (water loving) amino acids that should be clustered on the outside of the protein.



### Science Take-Out: From DNA to Protein Structure and Function (page 3)

#### B. From DNA to Protein – Record Sheet

**Step A.** Here is the sequence of bases in the DNA code for part of a gene DNA molecule

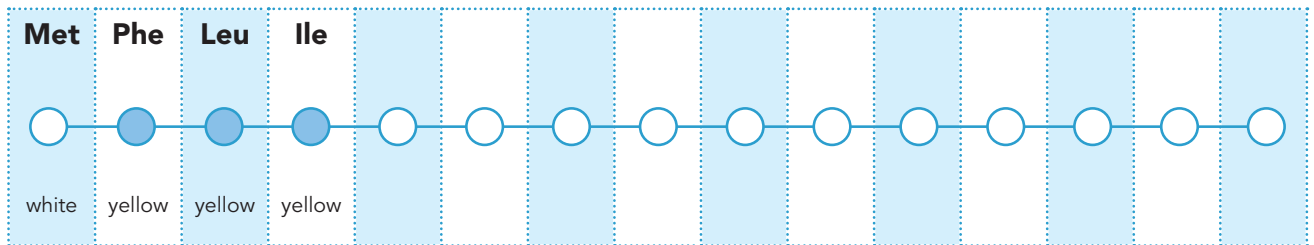
TAC	AAA	GAA	TAA	TGC	ATA	ACA	TTT	CAA	ACC	TCA	TCG	TTA	CTC	CCT
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

**Step B.** Transcribe the DNA and write the sequence of bases in the mRNA code RNA molecule

AUG	UUU	CUU	AUU											
-----	-----	-----	-----	--	--	--	--	--	--	--	--	--	--	--

**Step C.** Translate the mRNA and write the sequence of amino acids in the protein. Indicate the color associated with each of the amino acids in the circle below.

*Sequence of amino acids in protein – the primary structure of your protein model*



**Step D.** Fold your protein model into a 3-D shape.

Draw the secondary structure of your protein model	Draw the tertiary structure of your protein model	Draw the quaternary structure of your protein model





## Science Take-Out: From DNA to Protein Structure and Function (page 4)

### C. From DNA to Protein – Application To GAA

Acid alpha-glucosidase (GAA) is a protein molecule found in lysosomes. GAA processes excess glycogen being stored in the lysosome and converts it to smaller components of glucose. Pompe disease is a primarily a muscular disease caused by a build-up of glycogen in the lysosomes. If lysosomes cannot break glycogen down, they swell, displacing surrounding muscle cells. Additionally, the lysosomes can burst, releasing acidic components to the surrounding area and destroying muscle tissue. People with Pompe disease inherit a mutation in the DNA base code that carries the information for how to make the GAA protein. This DNA mutation leads to a change in the sequence of the amino acids for GAA. In Pompe disease, one amino acid in the GAA protein is incorrect.

1. To model the effect of a change in the amino acid sequence of a protein, replace one of the yellow beads on your protein molecule with a red bead. Follow the “protein folding rules” on page 2 to refold your protein model.
2. How does changing the amino acid sequence on your model affect the protein model?

The change in the shape in the GAA protein compromises the enzymatic function of the protein. Since enzymes have a particular shape and fit together with specific compounds, if the shape of the enzyme is altered, it will not be able to function properly.

3. Explain how the change in enzyme function affects the structure and function of lysosomes.
4. Explain how the change in lysosome structure and function results in the symptoms of Pompe disease.
5. Arrange the following phrases in order to indicate the sequence of events that would occur as a result of a gene mutation in the gene that codes for the protein acid alpha-glucosidase (GAA).
  - \_\_\_ Gene mutation (change in the DNA code in the gene for GAA)
  - \_\_\_ Change in the mRNA code
  - \_\_\_ Change in the amino acid sequence of the GAA protein
  - \_\_\_ Change in the structure or function of body cells or tissues
  - \_\_\_ Change in the shape of a GAA protein
  - \_\_\_ Change in the ability of the GAA protein to function properly
  - \_\_\_ Change in characteristics and symptoms of a genetic disease



## Science Take-Out: From DNA to Protein Structure and Function

1. To model the effect of a change in the amino acid sequence of a protein, replace one of the yellow beads on your protein molecule with a red bead. Follow the "protein folding rules" on page 2 to refold your protein model.

2. How does changing the amino acid sequence on your model affect the protein model?

Changes folding since different amino acids have different properties (i.e., attracted vs repelled) which then alters the shape or 3D structure of the protein

The change in the shape in the GAA protein compromises the enzymatic function of the protein. Since enzymes have a particular shape and fit together with specific compounds, if the shape of the enzyme is altered, it will not be able to function properly.

3. Explain how the change in enzyme function affects the structure and function of lysosomes.

The GAA enzyme is unable to break down glycogen in the lysosomes, causing glycogen to build up in the lysosome. As lysosomes become large and filled w/glycogen, the function of the entire cell is compromised.

4. Explain how the change in lysosome structure and function results in the symptoms of Pompe disease.

The growing lysosome displaces muscle cells. As the number of healthy muscle cells decreases, the integrity of the muscle tissue deteriorates, resulting in reduced muscle function. The compromised skeletal and cardiac muscles result in cardiac, diaphragm, and large motor movement difficulties.

5. Arrange the following phrases in order to indicate the sequence of events that would occur as a result of a gene mutation in the gene that codes for the protein acid alpha-glucosidase (GAA).

1 Gene mutation (change in the DNA code in the gene for GAA)

2 Change in the mRNA code

3 Change in the amino acid sequence of the GAA protein

6 Change in the structure or function of body cells or tissues

4 Change in the shape of a GAA protein

5 Change in the ability of the GAA protein to function properly

7 Change in characteristics and symptoms of a genetic disease

# Putting It All Together

## 5

### Vocabulary:

**Transcription:** DNA → RNA; During transcription, a DNA sequence is read by an RNA polymerase, which produces a complementary, antiparallel RNA strand. The RNA complement includes uracil (U) in all instances where thymine (T) would have occurred in a DNA complement.

**Translation:** RNA → Protein; In translation, messenger RNA (mRNA) produced by transcription is decoded by the ribosome to produce a specific amino acid chain, or polypeptide, that will later fold into an active protein.

**RNA:** Ribonucleic acid is one of the three major macromolecules (along with DNA and proteins) that are essential for all known forms of life. Like DNA, RNA is made up of a long chain of compo-

nents called nucleotides. Each nucleotide consists of a nucleobase, a ribose sugar, and a phosphate group. RNA directs the synthesis of proteins.

**Genotype:** The genotype is the genetic makeup of a cell, an organism, or an individual (i.e. the specific allele makeup of the individual). The genotype of an organism is the inherited instructions it carries within its genetic code.

**Phenotype:** A phenotype is the composite of an organism's observable characteristics or traits. Phenotypes result from the expression of an organism's genes as well as the influence of environmental factors and the interactions between the two.

**Reading frame:** a way of breaking a sequence of nu-

cleotides in DNA or RNA into three letter codons, resulting in a possibility of three reading frames in mRNA and six in double-stranded DNA (since have forward and reverse).

**Deletion:** removal of one or more nucleotides from a DNA sequence, which may alter the reading frame

**Insertion:** addition of one or more nucleotides in a DNA sequence, which may alter the reading frame

**Nonsense:** generally a single nucleotide change in the protein coding region that results in a different amino acid

**Missense:** generally a single nucleotide change in the protein coding region that results in a stop codon, causing the protein to be truncated

### KEY QUESTION(S):

- How can a definitive diagnosis be made?
- What testing can be performed?
- Can DNA predict protein structure and function and the resulting disease state?

### TIME ESTIMATE:

- Advanced Preparation: 30 minutes
- Student Procedure: 45 minutes

### LEARNING STYLES:

- Visual and kinesthetic.

## Lesson Summary:

Students perform a colorimetric assay on patient samples to determine % activity of GAA. They then perform a confirmatory test by comparing the patient DNA sequence to the reference and identifying any mutations. They will transcribe their sequence into mRNA and translate into amino acids. As an extension, once they have their amino acid sequence, they will use chenille stems and beads to create the amino acid chain and fold their protein. Comparing the mutant protein to the normal protein, students will infer the functionality of the mutant protein and predict the form of Pompe disease.

## Student Learning Objectives:

The student will be able to...

1. Discuss observed inheritance patterns caused by recessive mode of inheritance
2. Describe the basic process of DNA replication and the transmission of genetic information
3. Explain how mutations in the GAA gene may or may not result in phenotypic change
4. Model the basic processes of transcription and translation and how they result in the expression of the GAA gene to produce a protein
5. Explain how and why the genetic code is universal and is common to almost all organisms



### Implementation

**Note:** For simplicity, this activity only uses one DNA sequence, demonstrating mutation(s) present in one arm of the chromosome. Affected individuals are compound heterozygotes, so they actually inherit different mutations from their parents. You could easily adapt this lesson for more advanced students and present them with two sequences. You may also wish to tell the students we assume the other mutation to be a null allele, producing a non-functioning transcript. Additionally, students are only working with 45 nucleotides = 15 amino acids. The actual DNA sequence for the GAA protein is 20KB and contains 20 exons. This is too large to work with using paper and beads.

- Describe the basic molecular structures and primary functions of DNA and proteins (biological macromolecules)
- Construct proteins and amino acids structures. Explain the functions of proteins in living organisms. Relate the structure and function of enzymes.
- Explain the role of enzymes.
- Describe the function of models in science.

### Standards:

See table on page 11-13.

SC.912.L.14.6	SC.912.L.16.4	SC.912.L.18.1	SC.912.N.1.6
SC.912.L.16.2	SC.912.L.16.5	SC.912.L.18.4	SC.912.N.3.5
SC.912.L.16.3	SC.912.L.16.9	SC.912.L.18.11	

### Materials:

- DBS GAA Testing Kit, 1 per student group (8 patient cases are presented. For more groups, prepare duplicates.) Assembly instructions in Advanced Preparation.
- Optional extension activity: Science Take-Out: *From DNA to Protein Structure and Function*, 1 kit per student group (recommend pairs) – from previous lesson

Description	Source	Catalog #	Price
Phenol red indicating solution 0.2% aqueous solution (100mL)	Fisher Scientific <a href="http://www.fishersci.com">www.fishersci.com</a>	S71434	\$5.85
pH 3.0 buffer solution	Fisher Scientific <a href="http://www.fishersci.com">www.fishersci.com</a>	S06487	\$8.25
pH 10.0 buffer solution	Fisher Scientific <a href="http://www.fishersci.com">www.fishersci.com</a>	S06494	\$8.25
Filter paper Paper, 8cm x 10.5cm; 100 sheets	Fisher Scientific <a href="http://www.fishersci.com">www.fishersci.com</a>	88600	Pk of 100, \$49.98
Disposable transfer pipets (3mL total volume)	Fisher Scientific <a href="http://www.fishersci.com">www.fishersci.com</a>	13-711-7M	Pk of 500, \$49.60
Microfuge tubes (1.5mL), assorted colors, nonsterile	Fisher Scientific <a href="http://www.fishersci.com">www.fishersci.com</a>	02-682-556	Pk of 500, \$33.64
Sample collection cups	Science Take-Out <a href="http://www.sciencetakeout.com">www.sciencetakeout.com</a>	SC15	Pk of 50, \$5.00

### Supplemental:

3D models of GAA protein, glycogen, glucose (available from UF CPET or order separately from Biomolecular Modeling)

### Background Information:

#### Mutations

Pompe disease is an inherited disorder caused by a mutation of the acid alpha-glucosidase (GAA) gene, mapped to the long arm of chromosome 17. As an autosomal recessive disorder, Pompe only occurs when an individual inherits two mutant alleles, one from each parent. Most patients are compound heterozygotes, having inherited two different mutations. To date, almost 300 distinct GAA mutations have been identified, although not all are considered pathogenic. New mutations continue to be reported. The Pompe Center at the Erasmus University in Rotterdam, the Netherlands, maintains an up-to-date catalog of the GAA mutations.

## Genotype-Phenotype Correlations

In general, genotype-phenotype correlation is not well understood and significant clinical heterogeneity can exist among patients with similar or identical mutations. One well documented exception is the presence of two null mutations, resulting in a complete absence of GAA enzyme activity. This genotype results in very early disease presentation during infancy and severe, rapid disease progression. More studies, however, are needed in order to better understand genotype-phenotype relations, and a correlation cannot be assumed for any individual patient. Work is being done to further categorize mutations in an attempt to provide more targeted treatment. The progression of Pompe disease is highly variable and can be unpredictable, especially in patients with a later age of symptom onset. Researchers are still learning about the disease's molecular pathology and the factors—both genetic and environmental—that may influence disease progression and outcome.

## Diagnostics

If a clinician suspects Pompe disease, testing must be performed to confirm the diagnosis. The gold standard is fibroblast cultures or muscle biopsy to determine GAA activity and amount of glycogen present. This is an invasive technique, and sometimes mishandling causes inaccurate results. Additionally, cultures can take weeks to grow and measure activity. Recently, rapid testing methods have been developed to screen samples quickly and provide a diagnosis faster. This is particularly important when diagnosing infants. Dried blood spot samples can be assayed indirectly for GAA activity using fluorescence; mass spectrometry is also being evaluated for use as a diagnostic tool. Even with these testing methods, confirmatory tests must be done. Since Pompe is an inherited disease, genotyping not only confirms a diagnosis, but provides information for families, particularly those who wish to have children.

## Advance Preparation:

1. Copy *Student Worksheet: Clinical Lab Report* for each student.
2. Copy *Clinical Laboratory Protocol for the Diagnostic Testing of Pompe Disease* for each lab group
3. Copy *Patient Case Report* pages. Cut patient cases apart.
4. Collect Universal Genetic Code charts from Science Take-Out kits.
5. Optional extension activity – Reassemble Science Take-Out: *From DNA to Protein Structure and Function* kits.

Patient # and cases	Form of Pompe Disease	Colorimetric substrate	Colorimetric result
10031	Infant	500ul water	No color change (<1% GAA activity)
20032	Infant	500ul water	No color change (<1% GAA activity)
30033	Infant	500ul water	No color change (<1% GAA activity)
40034	Infant	500ul water	No color change (<1% GAA activity)
50035	Delayed-onset	500ul pH 3 buffer solution	Yellow (<20% GAA activity)
60036	Delayed-onset	500ul pH 3 buffer solution	Yellow (<20% GAA activity)
70037	Delayed-onset	500ul pH 10 buffer solution	Pink (<40% GAA activity)
80038	Delayed-onset	500ul pH 10 buffer solution	Pink (<40% GAA activity)

6. Label 8 ziptop bags with patient numbers.
7. Aliquot reagents:
  - a. 1.5ml water into (8) 1.5ml microfuge tubes. Label tubes PEB (Protein Elusion Buffer)
  - b. Place one tube PEB in each patient bag.
  - c. Label 8 microfuge tubes CS (colorimetric substrate).
  - d. Aliquot 500ul colorimetric substrate for each patient according to the chart above.
    - i. 4 tubes water (patients 1-4)
    - ii. 2 tubes pH 3.0 buffer solution (patients 5 and 6)
    - iii. 2 tubes pH 10.0 buffer solution (patients 7 and 8)
  - e. Place tube in corresponding patient bag.



**Implementation Note:** This lesson is written for 8 lab groups of 4 students each. It is preferable for each lab group to have two DNA to Protein kits; one that can be used to construct the normal protein and the other to construct the mutant protein. They can use one model from the previous lesson Part B. Students can then directly compare the structures.



### EXTENSIONS:

Use BLAST to have students search and compare GAA sequences. (Activity in development.)

<http://blast.ncbi.nlm.nih.gov/Blast.cgi>

The Protein Database (PDB) and Jmol can be used to show protein structures. (Activity in development.)

<http://www.rcsb.org/pdb/home/home.do>

<http://jmol.sourceforge.net/>

8. Prepare dried “blood” spots.
  - a. Cut filter paper into (8) 1 x 2 inch (2.5 x 5 cm) rectangles.
  - b. Using a disposable pipette, spot two drops of phenol red indicating solution on each filter paper rectangle, creating two “blood” spots.
  - c. Allow to dry.
  - d. Place 1 filter paper rectangle in each patient bag.
9. Prepare patient sequences (copy and cut apart)
  - a. Place patient sequence in corresponding patient bag.
10. Prepare GAA activity scale (8) (color copy, cut, laminate for each student group).
  - a. Place one GAA activity scale in each patient bag.
11. Assemble DBS GAA Testing Kit Contents in patient bags
  - Colorimetric Substrate (Specific to the patient, according to chart previous page – 1 per kit, labeled CS)
  - Protein elution buffer (1.5ml tubes of water, labeled PEB)
  - Patient dried “blood” spots
  - Patient sequence (specific to the patient)
  - GAA activity scale (1)
  - Collection cups (2)
  - Disposable pipets (2)
  - Scissors or single hole punch
  - Forceps (optional)

### Procedure with Time Estimates:

1. **(2 min)** Tell the students they are now going to take on the role of a laboratory technician. Patient samples have been submitted, and each lab team is to perform the tests and complete the lab report sheet to inform the requesting clinician of the results.
2. **(2 min)** Distribute *Student Worksheet: Clinical Lab Report* to each student.
3. **(2 min)** Ask students to work in groups of 2-4 (Eight groups recommended.)
4. **(2 min)** One member of the group should collect:
  - a. A patient DBS GAA Testing Kit (contains their patient samples as well)
  - b. *Clinical Laboratory Protocol for the Diagnostic Testing of Pompe Disease*
  - c. Universal Genetic Code chart
5. **(10 min)** Tell the students to follow the directions given for the DBS GAA Assay.
6. **(2 min)** Students should record their results on their lab sheet.
7. **(15 min)** Once students have finished the GAA Assay and cleaned up their station, they can continue completing their worksheet.
8. **Optional: (15 min)** Students can create a model of their patient’s protein using the DNA → Protein kit from the previous lesson. Instruct students to follow the directions on the kit lab sheet to create a model of their patient’s protein.
9. **(5 min)** As groups finish, ask them to add their mutation/protein/function information to the class *Grand Rounds Worksheet* chart. To avoid a bottleneck, you may prefer to project the teacher answer key during the next activity: Grand Rounds.

### Assessment Suggestions:

- Lab report sheet can be collected.

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### RESOURCES/REFERENCES:

Pompe mutation database: <http://cluster15.erasmusmc.nl/klgn/pompe/mutations.html>

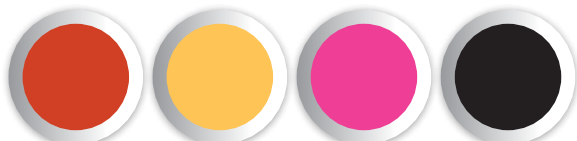
*Structural modeling of mutant -glucosidases resulting in a processing/transport defect in Pompe disease* Journal of Human Genetics (2009) 54, 324–330



# GAA Activity Assay Colorimetric Scale

Copy and cut. (Laminate for repeated use.)

## GAA Activity Assay Colorimetric Scale



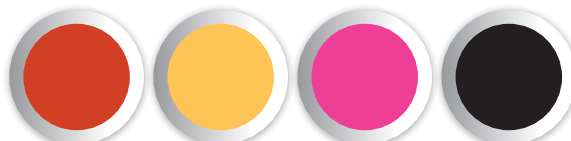
<1%  
GAA  
activity

<20%  
GAA  
activity

<40%  
GAA  
activity

>40%  
GAA  
activity

## GAA Activity Assay Colorimetric Scale



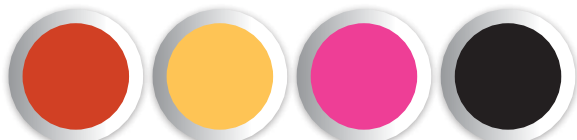
<1%  
GAA  
activity

<20%  
GAA  
activity

<40%  
GAA  
activity

>40%  
GAA  
activity

## GAA Activity Assay Colorimetric Scale



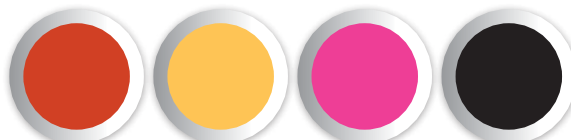
<1%  
GAA  
activity

<20%  
GAA  
activity

<40%  
GAA  
activity

>40%  
GAA  
activity

## GAA Activity Assay Colorimetric Scale



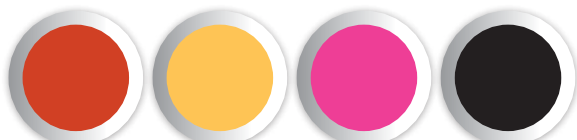
<1%  
GAA  
activity

<20%  
GAA  
activity

<40%  
GAA  
activity

>40%  
GAA  
activity

## GAA Activity Assay Colorimetric Scale



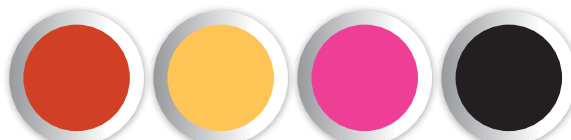
<1%  
GAA  
activity

<20%  
GAA  
activity

<40%  
GAA  
activity

>40%  
GAA  
activity

## GAA Activity Assay Colorimetric Scale



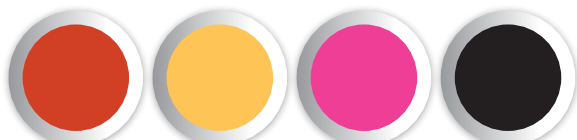
<1%  
GAA  
activity

<20%  
GAA  
activity

<40%  
GAA  
activity

>40%  
GAA  
activity

## GAA Activity Assay Colorimetric Scale



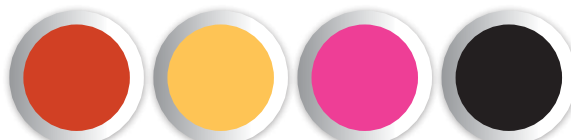
<1%  
GAA  
activity

<20%  
GAA  
activity

<40%  
GAA  
activity

>40%  
GAA  
activity

## GAA Activity Assay Colorimetric Scale



<1%  
GAA  
activity

<20%  
GAA  
activity

<40%  
GAA  
activity

>40%  
GAA  
activity



# Patient DNA Sequences

<b>Patient 10031</b>	DNA = TAC AAA GAA TAA TGC ATT ACA TTT CAA ACC TCA TCG TTA CTC CCT
<b>Patient 20032</b>	DNA = TAC AAA GAA TAA TGC ATA ACT TTT CAA ACC TCA TCG TTA CTC CCT
<b>Patient 30033</b>	DNA = TAA CAA AGA ATA ATG CAT AAC ATT TCA AAC CTC ATC GTT ACT CCC T
<b>Patient 40034</b>	DNA = TAC AAG AAT AAT GCA TAA CAT TTC AAA CCT CAT CGT TAC TCC CT
<b>Patient 50035</b>	DNA = TAC AAA GAA TGA TGC ATA ACA TTT CAA ACC TAA TCG TTA CTC CCC
<b>Patient 60036</b>	DNA = TAC AAA GAA TAA TGC ATA ACA TTT CAA ACC TCA TCG TTT ACT CCC T
<b>Patient 70037</b>	DNA = TAC AAA GAA TAA TGC ATA ACA TTT CAA ACC TCA TCG TTA CTT CCT
<b>Patient 80038</b>	DNA = TAC AAA GAA TAA TGC CTA ACA TTT CAA ACC TCA TCG TTA CTC CCT





# Patient DNA Sequences (page 1)

PATIENT 65	FORM OF POMPE DISEASE	COLORIMETRIC SUBSTRATE	COLORIMETRIC RESULT
10031	Infant	100ul water	No color change (<1% GAA activity)
	TAC AAA GAA TAA TGC ATT ACA TTT CAA ACC TCA TCG TTA CTC CCT AUG UUU CUU AUU ACG UAA UGU AAA GUU UGG AGU AGC AAU GAG GGA MET PHE LEU ILE THR Stop TYR → STOP 18A>T Point mutation causes NONSENSE MUTATION		
20032	Infant	100ul water	No color change (<1% GAA activity)
	TAC AAA GAA TAA TGC ATA ACT TTT CAA ACC TCA TCG TTA CTC CCT AUG UUU CUU AUU ACG UAU UGA AAA GUU UGG AGU AGC AAU GAG GGA MET PHE LEU ILE THR TYR Stop CYC → STOP 21A>T Point mutation causes NONSENSE MUTATION		
30033	Infant	100ul water	No color change (<1% GAA activity)
	TAA CAA AGA ATA ATG CAT AAC ATT TCA AAC CTC ATC GTT ACT CCC T INSERTION CAUSES FRAMESHIFT ELIMINATING START CODON (NO PROTEIN MADE)		
40034	Infant	100ul water	No color change (<1% GAA activity)
	TAC AAG AAT AAT GCA TAA CAT TTC AAA CCT CAT CGT TAC TCC CT AUG UUC UUA UUA CGU AUU GUA AAG UUU GGA GUA GCA AUG AGG GA MET PHE LEU LEU ARG ILE VAL LYS PHE GLY VAL ALA MET ARG BASE 6 DELETION (A) CAUSES FRAMESHIFT AND COMPLETELY DIFFERENT AA SEQUENCE		
50035	Delayed-onset	100ul pH 3 buffer solution	Yellow (<20% GAA activity)
	TAC AAA GAA TGA TGC ATA ACA TTT CAA ACC TAA TCG TTA CTC CCC AUG UUU CUU ACU ACG UAU UGU AAA GUU UGG ATU AGC AAU GAG GGG MET PHE LEU THR THR TYR CYC LYS VAL TRP ILE SER ASN GLY GLY  14A>G 4 <sup>th</sup> aa: ILE → THR (YELLOW BEAD → WHITE BEAD) 32C>A 11 <sup>th</sup> aa: SER → ILE (WHITE BEAD → YELLOW BEAD) 45T>C 15 <sup>th</sup> aa: CCT → CCC = NO CHANGE IN AA (silent mutation) Point mutations with change in amino acid = missense mutation		



# Patient DNA Sequences (page 2)

PATIENT #	FORM OF POMPE DISEASE	COLORIMETRIC SUBSTRATE	COLORIMETRIC RESULT
60036	<b>Delayed-onset</b>	<b>100ul pH 3 buffer solution</b>	<b>Yellow (&lt;20% GAA activity)</b>
	<p>TAC AAA GAA TAA TGC ATA ACA TTT CAA ACC TCA TCG TTt ACT CCCT            AUG UUU CUU AUU ACG UAU UGU AAA GUU UGG AGU AGC AAa UGA GGGA            MET PHE LEU ILE THR TYR CYC LYS VAL TRP SER SER LYS Stop</p> <p>INSERTION CAUSES FRAMESHIFT, ASN → LYS (WHITE → BLUE) AND STOP CODON            Missense and nonsense mutation</p>		
70037	<b>Delayed-onset</b>	<b>100ul pH 10 buffer solution</b>	<b>Pink (&lt;40% GAA activity)</b>
	<p>TAC AAA GAA TAA TGC ATA ACA TTT CAA ACC TCA TCG TTA CTT CCT            AUG UUU CUU AUU ACG UAU UGU AAA GUU UGG AGU AGC AAU GAA GGA            MET PHE LEU ILE THR TYR CYC LYS VAL TRP SER SER ASN GLU GLY</p> <p>GLY → GLU (YELLOW BEAD → RED BEAD)            42C&gt;T Point mutation with change in amino acid = missense mutation</p>		
80038	<b>Delayed-onset</b>	<b>100ul pH 10 buffer solution</b>	<b>Pink (&lt;40% GAA activity)</b>
	<p>TAC AAA GAA TAA TGC CTA ACA TTT CAA ACC TCA TCG TTA CTC CCT            AUG UUU CUU AUU ACG GAU UGU AAA GUU UGG AGU AGC AAU GAG GGA            MET PHE LEU ILE THR ASP CYC LYS VAL TRP SER SER ASN GLY GLY</p> <p>TYR → ASP (WHITE BEAD → RED BEAD)            16A&gt;C Point mutation with change in amino acid = missense mutation</p>		



# Clinical Laboratory Protocol for the Diagnostic Testing of Pompe Disease

## A. Acid Alpha-glucosidase Activity Assay

When a clinician suspects Pompe disease, the patient's blood is spotted on a piece of filter paper. The dry blood spot (DBS) can then be easily transported for testing of GAA activity.

### DBS GAA Testing Kit Contents

- Collection cups (2)
- Protein elution buffer (PEB)
- Colorimetric substrate (CS)
- Disposable pipettes (2)
- GAA activity scale
- Forceps
- Scissors or hole punch
- Patient information: DBS, DNA sequence, case reports



For lab safety, gloves should be worn during this assay.

1. Obtain your patient samples. Record your patient id number on your lab report.
2. Punch a circle or cut a 3mm square from the center of a dried blood spot (DBS). Use your forceps to place the sample in a collection cup.
3. Repeat step two, cutting from a different DBS and placing in a second collection cup.
4. Slowly add Protein Elution Buffer (PEB) to each collection cup until the sample is submerged (15-30 drops).
5. Allow to stand for **three** minutes.
6. Add 10 drops of colorimetric substrate (CS) to one collection cup.
7. Do not add substrate to the other cup. This is your negative control. *(The protein elution buffer will appear red-orange in the control as the proteins in the blood spot go into solution.)*
8. Allow to stand for **three** minutes.
9. Compare the resulting color to the GAA activity scale.
10. Record the results on the lab report.



# Clinical Laboratory Report

NAME	DATE
PATIENT ID #	

## A. Acid Alpha-glucosidase Activity Assay

- DBS GAA Colorimetric results
  - Record the color observed by DBS GAA colorimetric analysis \_\_\_\_\_
  - Record the %GAA activity indicated by colorimetric analysis \_\_\_\_\_

## B. Genetic Screening Analysis

A second confirmatory test is ordered when the GAA activity assay indicates Pompe disease. Genotyping is one such confirmatory test.

- Write your patient's sequence below and complete the chart.
- Circle the mutations (DNA, mRNA, and aa).

Ref sequence	DNA =	TAC	AAA	GAA	TAA	TGC	ATA	ACA	TTT	CAA	ACC	TCA	TCG	TTA	CTC	CCT
	mRNA =	AUG	UUU	CUU	AUU	ACG	UAU	UGU	AAA	GUU	UGG	AGU	AGC	AAU	GAG	GGA
	AA =	MET	PHE	LEU	ILE	THR	TYR	CYC	LYS	VAL	TRP	SER	SER	ASN	GLY	GLY
Patient	DNA															
	mRNA															
	AA															

## C. Interpret Your Results

- Predict the effect of the mutation(s) on the structure of the protein using the following scale:
  - No protein produced
  - Protein structure severely compromised
  - Some amino acid changes resulting in an altered protein structure
  - No change in the structure of the protein
- Support your answer using evidence from the lab report.



### CLINICAL LABORATORY REPORT (continued...)

3. Predict the effect of the mutation on the function of the protein and enzyme activity using the following scale:
  - a. No GAA enzyme produced
  - b. Minimal GAA enzyme produced and/or limited function
  - c. Some GAA enzyme produced although the function is reduced
  - d. Normal GAA enzyme produced and function is normal
  
4. Support your answer using evidence from the lab report.
  
5. The results of these two tests indicate a diagnosis. What form of Pompe disease do you suspect this patient has?
  - a. Infant
  - b. Delayed on-set
  - c. The patient does not have Pompe disease.
  
6. Complete the chart below for your patient.

PATIENT NUMBER	GAA ACTIVITY RESULTS COLOR AND %	MUTATION(S)	EFFECT ON PROTEIN STRUCTURE	PREDICTED FUNCTION

PATIENT NUMBER	AGE OF ONSET	SYMPTOMS	FORM OF POMPE DISEASE	TREATMENT

### D. Your Patient Case Report

Ask your teacher for your patient's case report. Summarize below.



# Grand Rounds – Class Data

Patient #	GAA Activity Results Color And %	Mutation(s)	Effect On Protein Structure	Predicted Function	Age Of Onset	Symptoms	Form Of Pompe Disease	Treatment
10031								
20032								
30033								
40034								
50035								
60036								
70037								
80038								



# Grand Rounds – Class Data

Patient #	GAA Activity Results Color And %	Mutation(s)	Effect On Protein Structure	Predicted Function	Age Of Onset	Symptoms	Form Of Pompe Disease	Treatment
10031	No color change (<1% GAA activity)	TYR → STOP 18A>T Point mutation causes Nonsense Mutation	Protein structure severely compromised	Minimal GAA enzyme produced and/or limited function	6.5 months	Heart Murmur Weak Muscles G-Tube	Infant	ERT
20032	No color change (<1% GAA activity)	CYC → STOP 21A>T Point mutation causes Nonsense Mutation	Protein structure severely compromised	Minimal GAA enzyme produced and/or limited function	5 months	Floppy Baby Respiratory Problems Physically Weak; Can't Sit Up, Little Head Control	Infant	ERT
30033	No color change (<1% GAA activity)	Insertion (A) Causes Frameshift Eliminating Start Codon	No protein produced	No GAA enzyme produced	3.5 months	Floppy Baby Murmur Ng Tube	Infant	Died Before ERT
40034	No color change (<1% GAA activity)	Base 6 Deletion (A) Causes Frameshift And Completely Different AA Sequence	Protein structure severely compromised	Minimal GAA enzyme produced and/or limited function	birth	Enlarged Heart Respiratory Distress Weak Muscles	Infant	Died Before ERT
50035	Yellow (<20% GAA activity)	14A>G 4th aa: ILE → THR 32C>A 11th aa: SER → ILE Missense mutation 45T>C 15th aa: CCT → CCC = No Change IN AA (silent mutation)	Some amino acid changes resulting in an altered protein structure	Some GAA enzyme produced although the function is reduced	7 years	Respiratory Problems Muscle Weakness	Delayed On-Set (Juvenile)	ERT Physical Therapy
60036	Yellow (<20% GAA activity)	Insertion Causes Frameshift, Asn → Lys And Stop Codon Missense and non-sense mutation	Some amino acid changes resulting in an altered protein structure	Some GAA enzyme produced although the function is reduced	16 years	severe fatigue and weakness	Delayed on-set (juvenile)	Bi-Pap at night special diet exercise program *waiting for ERT
70037	Pink (<40% GAA activity)	42C>T GLY → GLU Missense mutation	Some amino acid changes resulting in an altered protein structure	Some GAA enzyme produced although the function is reduced	~25 years	digestive issues decreased pulmonary function	Delayed on-set	high protein diet (limited carbs) exercise program physical therapy
80038	Pink (<40% GAA activity)	16A>C TYR → ASP Missense mutation	Some amino acid changes resulting in an altered protein structure	Some GAA enzyme produced although the function is reduced	27 years at onset; 31 years at diagnosis	weakness pain struggling to breath	Delayed on-set	Bi-Pap at night *waiting for ERT



# Patient Case Reports (page 1)

Case reports are adapted from patient stories on the AMDA website: <http://www.amda-pompe.org/index.php/main/patients/>. Please remind students these are actual cases and to treat these patients with utmost respect and dignity.

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## **PATIENT NUMBER: 10031**

Everything seemed to go well for the first few months of his life. Then a heart murmur was detected. After examination by ultra-sound, the doctor informed us that he has a very rare condition known as hypertrophic cardiomyopathy. Finding the cause of his hypertrophic cardiomyopathy was our next quest. A pediatric cardiologist who specializes in molecular medicine officially diagnosed our son with Pompe's disease at 6.5 months of age (3 months after the hypertrophic cardiomyopathy diagnosis).

Getting in the ERT clinical trial was our next step. After lots of phone calls and lots of finger crossing B was accepted into Clinical trial # AGLU01702 at the age of 8 months. It has been almost 2 years since his first infusion and we have seen a tremendous turn in his overall health in that time. He is not on any ventilation but does have a G-tube. The most important issue was his heart. The hypertrophic cardiomyopathy has regressed quite extensively, and its function is now completely normal. He has much better shoulder strength than he did at 1 year of age and has recently been able to push himself in his wheelchair. There are many minor improvements and his overall condition seems to be improving, however there are still persistent muscle groups that are continuing to elude therapy.

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## **PATIENT NUMBER: 20032**

Our son, the youngest of four children, was diagnosed with Pompe disease at 5 months of age. He had a very normal birth and development but was a floppy baby. He was first hospitalized at 6 months of age with RSV virus and pneumonia after which he was noticeably weaker than before. At 18 months of age he was again hospitalized several times with pneumonia. He suffered complete respiratory failure and was transported to the Medical Center Hospital about 60 miles from our home. He was trached, ventilated, and a G-tube for feeding was inserted.

At 5 years old, he is a very beautiful, bright, and loving child. His cognitive development is normal, but he is physically extremely weak. He has learned to speak using a speaking valve attached to his trach and is able to play with toys if his elbows are supported on a table. He cannot sit up at all without support and has very little head control. He can kick his legs and move his arms. He has his own lap top computer that he uses for learning and playing. He was finally able to start enzyme replacement therapy as part of a clinical trial at the age of 8.

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## **PATIENT NUMBER: 30033**

For the first 3 months of her life she was a normal baby – gaining weight, feeding well, kicking her legs, laughing and cooing. At about 3.5 months, she was not as active anymore and became fussy during feedings. About month later, we knew something was wrong. Her development had literally come to a halt. Her arms and legs had no muscle tone, she hadn't gained any weight in almost a month, and she appeared lethargic. The doctor in the ER listened to her heart and heard a noticeable murmur. After an X-ray, EKG and Echo, the cardiologist and four other doctors gave us the worst news of our lives. Our daughter had Pompe disease.

She was monitored closely, until the doctors felt that she was stable enough to come home. We had to learn how to give her feedings through an NG (Nasogastric) tube and administer her medication through the tube (she was on medicine for her enlarged heart).

They wanted her to start the Myozyme treatment immediately while she still had the strength to endure it. Unfortunately it was not meant to be. Over the next few days her health deteriorated. She had difficulty breathing and had no appetite. The cardiologist said that the disease was progressing rapidly. She was too weak for the enzyme treatment and would probably only live for a few days. They moved us to a small room off of the ICU. They started giving her morphine for the pain and increasing the dosage every few hours. She was struggling to breathe. Early that morning, my brave little girl passed away.





# Patient Case Reports (page 2)

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## PATIENT NUMBER: 40034

Although he looked like a beautiful and healthy 10lb 9oz baby boy, he had some problems with his heart being enlarged. On day one, before I had a chance to hold him, he would take his first flight to the local trauma center where he would spend the next 10 days fighting to breathe on his own. He was almost 2 months old when he got a bad cold that wouldn't go away. At that time I was told his heart had been large by some x-ray techs, but after testing by the heart doctor, I was told there were no worries. The months to follow were okay. He would get a cold fast, but it was that time of the year. He seemed to be holding his own.

Then he had trouble going to the bathroom, eating, and even being on his belly for longer than a minute. He couldn't roll over yet but he was still young. Everything else seemed fine. When the tests started coming back, there was a sure sign that something was very wrong. His liver function was down; his iron was very low, and he was considered malnourished.

One morning he got sick, and I took him to the doctor right away. He started having trouble breathing in the waiting room, and when we got back to see the doctor a few short minutes later, he was hardly breathing and almost blue. The next morning he was life-flighted once again. He was put on C-pap and started fighting real hard. The doctors told us they were pretty sure he had Pompe disease, and after a skin biopsy, they would know more. They told us it would take 6 weeks for the results. That evening I was visited by the genetic doctor and told about a study going on at a university. It was a long shot but our only shot. We started the paperwork and waited for the results.

He was given a feeding tube and a high calorie formula. He started moving his hands which he hadn't done in so long. The day the news came that he was being considered for the clinical trial, we celebrated. It was a terrible disease, but now treatment might be possible. He had one more step before the treatment: he had to be evaluated by the doctors at the university. Once there, we were told he was too sick and would not be accepted for the trial. I returned to his room. As he looked up at me with those big blue eyes, I knew his fight was over.

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## PATIENT NUMBER: 50035

Patient is age 14 and resides in India. Symptoms of Pompe were evident in his case when he was 7 years old and his condition progressed over the years. Diagnosis took quite a few years since adequate facilities were not available in India. He had an episode of Respiratory Failure Type II in Dec 2009 and since then has been on BiPAP support 8 hours a day when he sleeps. He cannot walk without support. He is on ERT of Myozyme, obtained through the Indian Charitable Access Program of Genzyme Corporation since his December 2009 respiratory episode. In addition to his ERT, he undergoes physiotherapy three times a week. He attends school and is in the 9th grade.

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## PATIENT NUMBER: 60036

At 16 years of age, the patient was an accomplished violinist and had just won a major regional competition when she came down with flu symptoms. Severe fatigue and weakness followed, and she could no longer attend school. A year later she was finally diagnosed with Pompe disease. Her condition continued to deteriorate and she was about to give up her violin studies. She traveled to North Carolina and New York to visit specialists recommended by AMDA. Under their guidance, she started using a Bi-Pap ventilator at night and went on a special diet and exercise program.

In the following year, she gained more than 15 pounds and regained some of her strength. As she could no longer commute long distances regularly, she moved 90 miles to live near a university hospital and her music activities. Today, she is finishing high school through a public school home tutor and continuing her musical pursuits. She is planning to go to college and waiting for the upcoming enzyme replacement therapy.



# Patient Case Reports (page 3)

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## **PATIENT NUMBER: 70037**

Towards the end of college I started seeing physicians concerning digestive issues. Six years later, and after a myriad of tests, I was diagnosed with Pompe. I am now in my early 30's and feeling better than I was when I was diagnosed.

I made changes to my diet and exercise routine which have dramatically improved my health. I try to stick to a high protein diet, and limit carbohydrates. I exercise a lot – walking, pilates, physical therapy, and strength training. Taking care of myself is akin to a part time job. I have to put my health first. Four years ago I added Myozyme (aka Lumizyme) to my treatment. Since that time, my pulmonary function has stabilized, and in combination with all my physical therapy I have gained strength. Myozyme is not a cure, but over the long term I feel like it has made all the other work that I do much more beneficial. I even started skiing again!

The emotional challenge of a diagnosis like Pompe's has been tough. I feel cheated out of the life I always imagined growing up. It's difficult not knowing the course that my disease will take in the future. All I can do is try to work hard every day and try not to let Pompe run my life. I spend time relaxing at home, enjoying the outdoors, hanging out with friends and family, and traveling.

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## **PATIENT NUMBER: 80038**

I was diagnosed at the age of 31. I'd been having problems with weakness and pain for around 4 years. It was only during summer 2009 that things got worse, and I noticed how much it was affecting my day to day functioning and that I was struggling to breathe. I'd seen various general practitioners over the years who had told me I was unfit and needed to 'exercise more' and related my problems to life stresses.

I was referred to a neurologist, and then admitted to hospital for tests in, where I was diagnosed with Limb-Girdle Muscular Dystrophy. However, a muscle biopsy and further tests for LGMD came back negative. My consultant then suspected Pompe disease and it was confirmed through laboratory testing.

I'm on BiPap at night which has made a positive difference as I have more energy during the day and no longer have morning headaches. I'm currently waiting an appointment to enroll in ERT. I still work full time – my job is very busy and can be stressful and physically demanding at times but I really enjoy it.

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# Grand Rounds

## 6

### Vocabulary:

**Grand rounds** are an important teaching tool and ritual of medical education and inpatient care, consisting of presenting the medi-

cal problems and treatment of a particular patient to an audience consisting of doctors, residents and medical students. The patient was traditionally present for the

round and would answer questions; grand rounds have evolved with most sessions now rarely having a patient present and being more like lectures.

### Lesson Summary:

Building on the previous activity, students will present a summary of their patient case along with the lab results they found in a grand rounds fashion. This activity allows students the opportunity to read actual patient cases and work together to summarize the findings. When they present their summary to the class, each member of the group is responsible for presenting part of the case.

### Student Learning Objectives:

The student will be able to...

1. Explain the significance of genetic factors to health from the perspective of the individual.
2. Discuss recessive inheritance.
3. Discover how mutations in the DNA sequence may result in phenotypic changes and Pompe disease.
4. Explain the basic processes of transcription and translation and how they result in the expression of the GAA gene.
5. Describe the basic structure and function of DNA and protein.
6. Create proteins and amino acids structures. Explain the function of proteins in living organisms. Relate the structure and function of enzymes.

### Standards:

See table on page 11-13.

SC.912.L.14.6	SC.912.L.16.4	SC.912.L.18.1	SC.912.L.18.11
SC.912.L.16.2	SC.912.L.16.5	SC.912.L.18.4	

### Materials:

- *Grand Rounds Worksheet*, one per student
- *Optional: Grand Rounds Worksheet Poster* or overhead for students each student group to record data

### KEY QUESTION(S):

- How does Pompe disease present in different individuals?
- Is there a clear genotype/phenotype correlation for all forms of the disease?
- Are there common, predictive symptoms among the different forms of Pompe disease?

### TIME ESTIMATE:

- Advanced Preparation: 30 minutes
- Student Procedure: 45 minutes

### LEARNING STYLES:

- Visual and auditory



Part of the dilemma with diagnosing and treating Pompe disease is the multitude of different symptoms, particularly in patients developing symptoms later in life. While some mutations and consequent genotypes are clearly deleterious and often result in the most severe form of Pompe disease (infant or classic), other mutations present differently in different patients. Almost 300 different mutations have been identified to date.

## Background Information:

Part of the dilemma with diagnosing and treating Pompe disease is the multitude of different symptoms, particularly in patients developing symptoms later in life. While some mutations and consequent genotypes are clearly deleterious and often result in the most severe form of Pompe disease (infant or classic), other mutations present differently in different patients. Almost 300 different mutations have been identified to date.

The case histories that the students work from are adapted from actual stories given by parents and patients combined with information published regarding specific forms and associated phenotypes. The cases are therefore not completely accurate as they are several stories combined to allow students to put themselves in the place of the patients, families, and doctors. This will allow them to identify with the patient groups as they move through this unit ending with a gene therapy class discussion.

## Advance Preparation:

1. Make copies of *Grand Rounds Worksheet* for students.

## Procedure and Discussion Questions with Time Estimates:

1. **(2 min)** Ask students to reassemble with their lab group and take out their *Clinical Lab Report* from the previous day.
2. **(5 min)** Allow student groups to read and discuss the case and complete their *Grand Rounds Worksheet* with their patient's information as needed. While waiting for all groups to finish, encourage students to copy the patient data from the class chart to their *Grand Rounds Worksheet*.
3. **(3 min)** Read the definition of grand rounds. Review what grand rounds are and the purpose.
4. **(2 min)** Tell the students they will have 3 minutes to present their case in the format of grand rounds. They essentially need to complete the class chart and explain their results and if they are in keeping with the patient case report. The science must be presented accurately and in keeping with the ethical code of conduct for researchers and clinicians, and utmost respect for the patient. If desired, you could assign roles to each of the team members as below:
  - a. share lab results from the previous day,
  - b. discuss the mutation(s) present and how they affect the protein,
  - c. summarize the case (or read the case report),
  - d. indicate what form of Pompe the patient has and possible treatment options.
5. **(3 min)** Allow the first group 3 minutes to present their case. Introduce each group by the patient number.
6. **(25 min)** Repeat step 7 with the remaining groups.
7. Instruct the students to complete the questions on the back of their *Grand Rounds Worksheet* for homework.

## Assessment Suggestions:

- Collect *Grand Rounds Worksheet*.



# Grand Rounds Worksheet (page 1)

NAME \_\_\_\_\_

DATE \_\_\_\_\_

HOME GROUP MEMBERS \_\_\_\_\_

Patient #	GAA Activity Results Color And %	Mutation(s)	Effect On Protein Structure	Predicted Function
10031				
20032				
30033				
40034				
50035				
60036				
70037				
80038				



# Grand Rounds Worksheet (page 2)

Patient #	Age Of Onset	Symptoms	Form Of Pompe Disease	Treatment
10031				
20032				
30033				
40034				
50035				
60036				
70037				
80038				



# Grand Rounds Worksheet (page 3)

NAME

DATE

PATIENT NUMBER

1. Does your case report match your laboratory results? Why or why not?
2. Identify three similarities between the patients.
3. Identify three differences between the patients.
4. Does there seem to be a relationship between the DNA sequence (genotype) and the symptoms of the disease (phenotype)? If so, which patients have the strongest correlation?
5. What is the most common form of treatment for Pompe patients?
6. Can you describe any other experimental therapies that could be investigated?
7. Imagine a patient received treatment which allowed glycogen to be broken down in the lysosome, preventing further damage to the body. Is there any way to repair the damage that has already occurred?





# Exploring Gene Therapy

## 7

### Clinical Trials through Webquest and Role Play

#### Vocabulary:

##### Enzyme replacement

**therapy:** a medical treatment replacing an enzyme in patients in whom that particular enzyme is deficient or absent. Usually this is done by giving

the patient an intravenous (IV) infusion containing the enzyme. Enzyme replacement therapy does not “treat” the underlying disease, only the symptoms.

##### Pharmacological chaperone:

Small molecules that selectively bind to and stabilize proteins in cells, leading to improved protein folding and trafficking, and increased activity.

#### Lesson Summary:

In this lesson, students will learn about the benefits, dangers, and ethical dilemmas associated with gene therapy clinical trials through a webquest. They will then participate in a role play, depicting various stakeholders at an RAC meeting to decide the fate of a gene therapy trial. This activity serves as a wrap-up to the entire unit.

#### Student Learning Objectives:

The student will be able to...

1. Evaluate the impact of biotechnology on the individual and on society through role play.
2. Critique the ethical and morals standards of the stakeholders involved in conducting gene therapy trials.
3. Synthesize an educated stance on the controversial topic of gene therapy.
4. Explain the significance of genetic factors to health from the perspective of both individual and public health.
5. Describe how viruses transfer genetic material between cells and the role of this process in biotechnology.
6. Discuss how basic DNA technology is used to construct recombinant DNA molecules.
7. Describe the basic molecular structures and primary functions of DNA and proteins.
8. Recognize that the strength or usefulness of a scientific claim is evaluated through scientific argumentation, which depends on critical and logical thinking, and the active consideration of alternative scientific explanations to explain the data presented.
9. Explain how scientific knowledge and reasoning provide an empirically-based perspective to inform society’s decision making.
10. Weigh the merits of alternative strategies for solving a specific societal problem by comparing a number of different costs and benefits, such as human, economic, and environmental.

**Standards:** See table on page 11-13.

SC.912.L.14.6	SC.912.L.16.10	SC.912.L.18.1	SC.912.N.4.1
SC.912.L.16.7	SC.912.L.16.12	SC.912.N.1.3	SC.912.N.4.2

#### ? KEY QUESTION(S):

- What is gene therapy?
- What are the pros and cons associated with gene therapy?
- Is conducting gene therapy trials on humans ethical?

#### 🕒 TIME ESTIMATE:

- Advanced Preparation: 10 minutes
- Student Procedure:  
Day 1: 50 Minutes (if gene therapy webquest is completed in class)  
Day 2: 30 Minutes  
Day 3: 50 Minutes

#### 🎧 LEARNING STYLES:

- Visual, auditory, kinesthetic



Gene therapy is the use of DNA as a pharmaceutical agent to treat disease. It derives its name from the idea that DNA can be used to supplement or alter genes within an individual's cells as a therapy to treat disease.

## Materials:

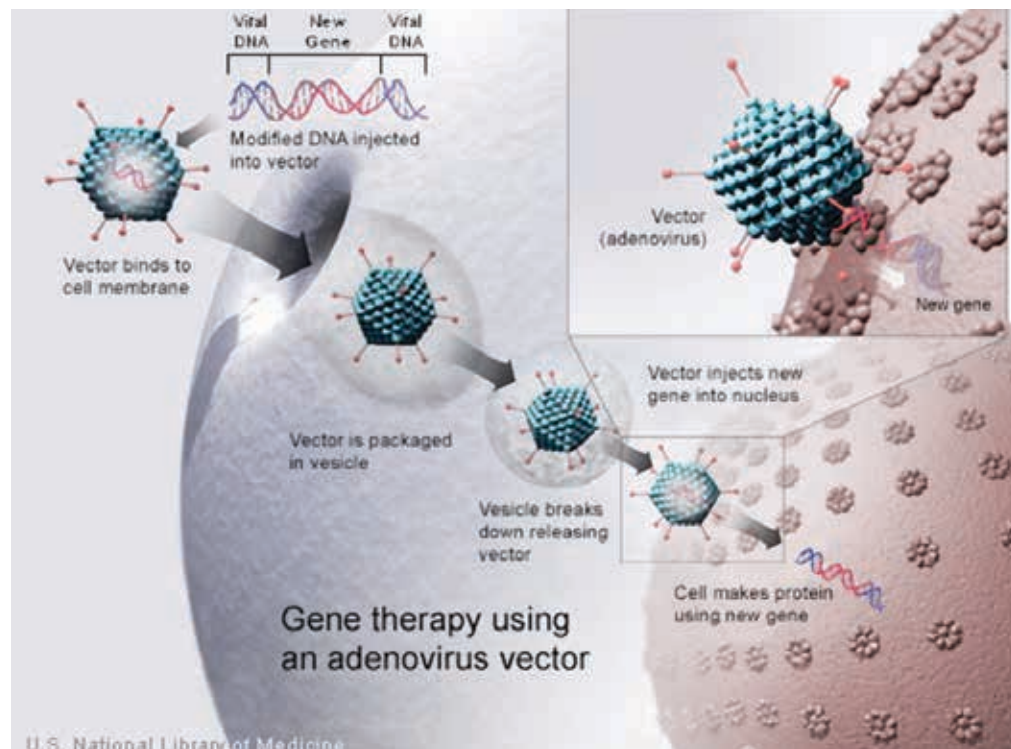
- Student gene therapy webquest pages (1 per student)  
Pages 10-13 of pdf located at <http://teach.genetics.utah.edu/content/tech/genethrapy/Exploring%20Gene%20Therapy%20.pdf>
- Student Handout: *Gene Therapy Role Play* (1 per student)
- Role Play Cards (1 per student in each stakeholder group)
- Student Worksheet: *Gene Therapy Role Play* (1 per student)
- Markers (1 per stakeholder group)
- Cardstock (1 per stakeholder group, to make name plate)

## Background Information:

Gene therapy is the use of DNA as a pharmaceutical agent to treat disease. It derives its name from the idea that DNA can be used to supplement or alter genes within an individual's cells as a therapy to treat disease. The most common form of gene therapy involves using DNA that encodes a functional, therapeutic gene in order to replace a mutated gene. In gene therapy, DNA that encodes a therapeutic protein is packaged within a "vector", which is used to get the DNA inside cells within the body. Once inside, the DNA becomes expressed by the cell machinery, resulting in the production of therapeutic protein, which in turn treats the patient's disease.

Early work with gene therapy began in the 1970s as researchers started developing the idea. The first clinical trials began in the 1990s. Gene therapy has suffered major setbacks, with the death of patients in two separate trials, resulting in very tight control of the approval and reporting process.

In 2010, researchers at the University of Florida began enrolling participants for a Phase I/II gene therapy clinic trial. Scientists have incorporated the correct gene to produce the enzyme GAA into an adeno-associated virus, which already exists in most people, and during the clinical trial, inject it into each patient's diaphragm. The intent is to "infect" cells of Pompe patients with the genetic machinery they have been missing since birth. If successful, this method would not be a cure for Pompe disease, but could drastically increase the quality of life for people with the disease.



## Advance Preparation:

(10 min)

- Reserve computer lab if completing webquest during class time.
- Print and cut out role play cards.

**Implementation Note:** While this role play is loosely based on the proceeding at the RAC meetings, the role card information is fictional. In particular, the statement about the death of animal models is not published data. This has been added to cause the students to realize animal models are used in early phases of research and often there are losses while dosing curves are determined. Additionally, pharmaceutical companies are businesses. While they fund many clinical trials, it is to promote their own product for the good of the business. They do not have a place at the scientific hearing to determine if clinical trials should begin. The RAC does exist, but the meetings are not open to all members of the public to plead their case. The meetings are to determine if the science is sound, the procedure shown to be safe, and if the investigator is competent to conduct a human trial.

There is not much controversy with somatic gene therapy, so this activity shouldn't lead to harsh words or disruptive behavior. If your students are unaccustomed to role play or engaging in socioscientific discussions, this role play will help by engaging them in a relatively uncontroversial topic. Ethics is a vital component of medicine and this activity should open the door for students to engage in more difficult topics such as germ line gene therapy. Our students today will be faced with these issues in the very near future.

## Procedure and Discussion Questions with Time Estimates:

Webquest (50 min if completed in class)

1. The webquest from the Genetic Science Learning Center is a great introduction to gene therapy. They provide a complete lesson plan on their website (<http://teach.genetics.utah.edu/content/tech/genetherapy/Exploring%20Gene%20Therapy%20.pdf>). The webquest could be assigned for homework before introducing the role play or completed in the school computer lab. If completing during class, allow 45 minutes.

*\*Implementation note: the companion teacher website has answers listed. These are accessible to anyone, even the students. There is a vector worksheet with the activity that you may wish to omit. For the purposes of this lesson, students only need to know generally that there are different vectors used to target different cells and deliver different genes. This is covered on the primary worksheet which accompanies the webquest.*

Introduction to Role Play (30 min)

1. The class period before the role play should be used to introduce and summarize the role play activity that the students will be conducting the next day.
2. Ensure all students have a general understanding of gene therapy and all aspects of Pompe disease including the cause, symptoms, and treatment options for patients. This can be accomplished by reviewing the gene therapy webquest answers and relating it to what they have learned about Pompe or briefly lecturing on the concept. Very brief presentation from PBS DNA website could be used in lieu of a short ppt to introduce gene therapy if webquest not done: [http://www.pbs.org/wnet/dna/pop\\_gene\\_therapy/index.html](http://www.pbs.org/wnet/dna/pop_gene_therapy/index.html). Additionally, part of the webinar by Dr. Byrne, listed below, could be shown to the students.
3. Divide the students into 6 groups. Each group will represent a stakeholder group during the round-table discussion. One group will represent the RAC committee. For simplicity, have an odd number of students in this group so their vote does not end in a tie.
4. Provide each group with their respective role card which details their stakeholder group's stance on implementing gene therapy clinical trials in an effort to find a better treatment for Pompe disease. At this time, distribute the *Student Handout: Gene Therapy Role Play*.



If your students are unaccustomed to role play or engaging in socioscientific discussions, this role play will help by engaging them in a relatively uncontroversial topic. Ethics is a vital component of medicine and this activity should open the door for students to engage in more difficult topics such as germ line gene therapy. Our students today will be faced with these issues in the very near future.



## EXTENSIONS:

### Activities

Have the student consider gene therapy with germ cells. The role play involves gene therapy of somatic cells. What if a patient is treated with gene therapy successfully and 20 years in the future the patient would like to have children? In that case, the sperm or egg would need to be genetically manipulated to correct the mutant GAA allele. Should this be permitted?

### Literature

Short essay [Gene Therapy: Ethical and Social Issues](#) to prompt student discussion of modifying germ cells. <http://academicdepartments.musc.edu/humanvalues/pdf/gene-therapy.pdf>

5. Have each stakeholder group create a name plate that they will display in front of their group during the round-table discussion.
6. Allow the remaining time (approximately 15 minutes) for the groups to:
  - a. Review the student handout/ become acquainted with the role play scenario and procedures.
  - b. Read their role card and discuss how they will portray their group during the role play

## ROLE PLAY DAY:

### Role Play Activity (50 Min)

- Arrange the student desks in a way that all the stakeholder groups are able to see one another. A circle/U-shape is recommended.
- Follow the procedures outlined on the *Student Handout: Gene Therapy Role Play* to conduct the role play. Note: The teacher assumes the role of a moderator in this role play activity.
- Pass out the *Student Worksheet: Gene Therapy Role Play* to the students. This worksheet should be completed for homework.

## Assessment Suggestions:

- *Student Worksheet: Gene Therapy Role Play*

### RESOURCES:

Gene therapy web quest:  
Teacher pages: <http://teach.genetics.utah.edu/content/tech/genetherapy/Exploring%20Gene%20Therapy%20.pdf>  
Student pages: <http://learn.genetics.utah.edu/content/tech/genetherapy/>

Very brief presentation from PBS DNA website (could be used in lieu of a short ppt to introduce gene therapy if webquest not done): [http://www.pbs.org/wnet/dna/pop\\_gene\\_therapy/index.html](http://www.pbs.org/wnet/dna/pop_gene_therapy/index.html)

NIH Curriculum Supplement Series: Bioethics <http://science.education.nih.gov/customers.nsf/HSBioethics.htm>

University of Florida News: <http://news.ufl.edu/2010/01/26/pompe-2/>

University of Florida: <http://www.peds.ufl.edu/research/teams/byrne.asp>  
Government Clinical Trials Website: <http://www.clinicaltrials.gov>

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Webinar by Dr. Byrne on gene therapy clinical trial, presented to AMDA: [http://www.amda-pompe.org/index.php/main/webinar/diaphragm\\_delivery\\_of\\_recombinant\\_adeno-associated\\_virus\\_acid\\_alpha-glucosi](http://www.amda-pompe.org/index.php/main/webinar/diaphragm_delivery_of_recombinant_adeno-associated_virus_acid_alpha-glucosi)

Department of Energy Human Genome Project [http://www.ornl.gov/sci/techresources/Human\\_Genome/medicine/genetherapy.shtml](http://www.ornl.gov/sci/techresources/Human_Genome/medicine/genetherapy.shtml)

First-hand account and discussion of the Penn State / Jessie Gelsinger gene therapy trial by Dr. James Wilson <http://mediasite.video.ufl.edu/mediasite/SilverlightPlayer/Default.aspx?peid=aa10ba9ce4bf4efe9d082b1237a869c61d>

Journal articles:

Spinal Delivery of AAV Vector Restores Enzyme Activity and Increases Ventilation in Pompe Mice <http://amdapompe.ehclients.com/downloads/publications/byrne.pdf>

Pompe disease gene therapy <http://amdapompe.ehclients.com/downloads/publications/Byrne-HMG2011.pdf>

Virus Vector Delivery to Murine Diaphragm <http://amdapompe.ehclients.com/downloads/publications/VirusDeliveryDIAPHRAGM.pdf>

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## Gene Therapy Role Play

The concept of “fixing” someone’s genes to a cure genetic disease once seemed unattainable and like something out of a science fiction movie, but the possibility is much closer than we ever imagined.

The novelty of this grand idea, however, was tarnished when an eighteen year old boy died in a gene therapy clinical trial in 1999. Since this incident, there has been much scrutiny about the safety and ethics behind gene therapy. Now, gene therapy clinical trials are monitored more closely than ever by the Recombinant DNA Advisory Committee (RAC) which advises the National Institute of Health (NIH). The RAC reviews human gene transfer research approaches. Protocols that raise any particularly important scientific, safety or ethical considerations are discussed by the RAC at one of its quarterly public meetings. It can take years to have a protocol approved and clinical trials to start.

Today we are at one of the RAC’s quarterly meetings in Bethesda, Maryland to discuss if scientists at the University of Florida should be granted permission to conduct gene therapy clinical research trials on patients with Pompe disease. Here today we have a diverse group of representatives to share their professional and personal opinion on the topic. At the conclusion of today’s meeting a vote will be conducted to determine if the clinical trial should be allowed.

### RAC Meeting Rules:

- You must be seated with all the members of your stakeholder group while the meeting is in progress.
  - A group member must raise their hand to indicate they would like to speak. You must then wait to be recognized by the moderator (your teacher).
  - Everyone must be respectful and listen when a stakeholder group is sharing their position. Any individual not in accordance with these rules will be asked to leave.
1. You will have 5 minutes to meet with your fellow group members. This time should be used to find a place to sit and set up your name plate. Also, use this time to determine one speaker for your case.
  2. **(10 min)** Once the meeting has begun, each group will be permitted 2 minutes to introduce their group and provide a brief synopsis on their position. The

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Today we are at one of the RAC’s quarterly meetings in Bethesda, Maryland to discuss if scientists at the University of Florida should be granted permission to conduct a gene therapy clinical research trial on patients with Pompe disease.

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moderator (your teacher) will call upon your group when it is time to speak. The moderator will select a group to go first and move in a clockwise fashion from that group on.

3. **(10 min)** The RAC committee members will be given a chance to ask one question of each group. The moderator will call on speakers who raise their hands.
4. **(5 min)** Intermission
  - a. Part 1: During this intermission time stakeholders are allowed to interact with each other and learn more about the motives and ethical values of the other groups. Individuals may want to share information they learned in previous lessons.
  - b. Part 2: Using the remaining time to rejoin your group and share any new information you have learned about the other stakeholder groups and formulate your closing argument.
5. **(10 min)** Meeting will commence. Each group will be given a 2 minute time period to present a closing statement. The groups will be called on in the order they were at the beginning of the meeting.
6. **(5 min)** The RAC group will have time to confer and reach a decision. Each member of the RAC will be allowed one vote: either **for** or **against** gene therapy clinical trials.
7. The moderator will call on the RAC to reveal its decision. The RAC spokesperson will indicate if your group is for or against clinical trials starting. Indicate what evidence led to the decision. If the vote is against, recommendations should be given (what is needed to show gene therapy for Pompe disease is safe and effective in human patients).



## Role Play Cards (page 1)

**CLINICIANS.** Since Pompe disease is so rare and finding a doctor who is exclusively specialized in treating patients with the disease is nearly impossible your group is made up of a variety of doctors. You have a neurologist, pulmonologist, cardiologist, oncologist, registered dietician, physical therapist, and an entire staff at a cardio-pulmonary rehab gym. Each of you attempts to improve your Pompe patient's quality of life of by relieving some of their symptoms. Despite your varying specialties you must collaborate to determine what the best treatment options are for your patients prior to implementing them. You are eager to hear and learn more about the possibilities of gene therapy clinical trials for Pompe disease and have a long list of questions to ask the researchers about the safety of this procedure. You are a strong supporter of translational research but want to ensure your patients will be safe. At the meeting you ask the principal investigator and his research team questions about their laboratory work and results thus far.

**PATIENTS WITH POMPE DISEASE.** Your group is composed of individuals that have been clinically diagnosed with Pompe Disease. You range in age from 3- 16 years old and are all experiencing a variety of symptoms associated with the disease including muscle weakness (cardiac and skeletal), complications walking and breathing, and organ enlargement. For many of you the disease is progressing at a fast rate and you now are confined to a wheelchair and require a respirator. You are aware that these symptoms will become more severe over time and will ultimately shorten your life. The possibility of being a participant in a gene therapy clinical trial that may reduce the burdensome symptoms that you are experiencing and extend your life expectancy is very exciting but also scary. You are nervous about participating in a clinical research study, but see few alternatives for treatment. You are unsure if you should participate in the study and seek your parents and doctors for guidance on this issue.

**PARENT OF PATIENTS WITH POMPE.** Everyone in your group has at least one child with Pompe disease. You are constantly conducting searches seeking new scientific findings and information on the disease and actively fundraising to provide monetary assistance for scientists researching Pompe disease. Despite your enthusiasm for translational research you are still hesitant to allow your child to participate in the study without being fully informed on the potential for negative reactions in response to the treatment. You are aware, that your child already has a decreased lifespan, but are nervous about complications that may arise during the trial that could end their life even more prematurely. To make matters worse you recently heard about a teenager who died due to complications associated with a gene therapy clinical trial he was participating in. You want reassurance from the doctors, nurses, and researchers before you can comfortably encourage your child to participate in a clinical trial. At the RAC meeting you will ask the principle investigator to share the results of all the gene therapy trials that were conducted on animals in the laboratory.



## Role Play Cards (page2)

**PRINCIPLE INVESTIGATOR.** As the principle investigator you hold a very prestigious position that also comes with great responsibility. You manage your group of researchers in the laboratory, monitor the progress of the clinical trials, and represent the University of Florida. You dedicated your career to researching the genetic mechanisms of Pompe disease and have been relentless in your quest to find a treatment for this rare condition. Along the way you have encountered difficulty obtaining funding to continue your research. Finally, however, you think you have it! You know that you will have to convince the RAC, and the families and patients with Pompe that your treatment procedure is safe and will work. At the meeting you must present evidence that you performed successful gene therapy trials on an animal model in the laboratory. You share that you have had some deaths related to the treatment; however, this occurred only when the mice were administered a treatment dosage 20 times higher than what they patient would be given. You reassure the panel that when the dosage that would be used in the human trails was administered it was tolerated by all the animal models. You also reassure all parties that each patient will be assessed prior to treatment to ensure they meet the requirements and health conditions necessary to be admitted into the trial.

**PHARMACEUTICAL COMPANY.** Your group represents a successful pharmaceutical company that is very interested in funding and patenting the principle investigator's work. You have previously been in contact with him and his research team. He has submitted his research findings to your company and you are optimistic that his treatment will work. While your company produces many different types of medicines to cure common diseases and conditions, you are allured by the potential to have your company's name associated with a treatment that can remedy Pompe disease even better than enzyme replacement therapy. At the RAC meeting you are interested to see how the panel and other stakeholders react when discussing the implementation of these gene therapy clinical trials. Overall, you are in strong support of clinical trials starting.

**RECOMBINANT DNA ADVISORY COMMITTEE (RAC).** Your group advises the National Institute of Health (NIH). The RAC reviews human gene transfer research approaches. Protocols that raise any particularly important scientific, safety or ethical considerations are discussed by the RAC at one of its quarterly public meetings. It can take years to have a protocol approved and clinical trials to start. You are all experts in the recombinant DNA research and clinical applications and understand the great responsibility you have as you determine the fate of a potential life saving therapy. You must also weigh the potential harm that may be done if the technology is not ready. Patient lives are at stake. Although this is a devastating disease, you cannot allow humans to be used an experimental manner that might cause more damage. You are interested to hear from the patients and parents who live with the disease everyday and learn more about their quality of life. You also are very impressed with the lead investigator and the careful experimental approach he has taken thus far. Not only is he a researcher, but he is also a leader in the clinical care of Pompe patients.



# Gene Therapy Role Play

NAME \_\_\_\_\_

DATE \_\_\_\_\_

*Instructions:* This worksheet is to be completed individually following the Gene Therapy Role Play activity. Use your stakeholder card and information you learned at the meeting to answer the following questions.

1. What stakeholder group did you represent?
2. What was your group's position on allowing gene therapy clinical trials to begin?
3. Explain if you personally agree/ disagree with the beliefs and ethical standards of your stakeholder group.
4. Imagine if you had a moderate case of Pompe Disease. Would you enroll yourself in a gene therapy clinical study? Explain the thought process behind your decision.
5. The clinical trial under consideration in this activity only affected somatic cells. Therefore, any modification to the individual would not be passed on to offspring. Consider the case 25 years in the future. One of our Pompe patients has successfully been treated with gene therapy and is living a very normal, happy life. He is married to a wonderful person, and they long to start a family. However, the man will pass mutations for Pompe along to his children. His wife has been tested and carries some of the mutations as well, although she does not exhibit symptoms of Pompe disease. What is your position on germ line (inheritable modification to sperm or egg) gene therapy to correct the mutant GAA gene? Are you for or against? Explain thoroughly your position on the back.



# Extension Activity

8



**SUBJECT:**

- Biology, Anatomy and Physiology

**GRADE**

- 10th -12th/ Honors or Regular

## Movie:

Extraordinary Measures. Dir. Tom Vaughan. Perf. Brendan Frasier, Harrison Ford, and Keri Russel. CBS Films, 2010. (Run time: 106 minutes)

## Film Summary:

Based off the book *The Cure*, “Extraordinary Measures” retells the true story of two parents who were determined to save their children from Pompe Disease, an extremely rare and potentially fatal genetic disorder. The film addresses a variety of topics relevant to biology including the nature of science, genetic disorders, and scientific protocol.

## Additional Materials:

Anand, Geeta. *The Cure*. New York, NY: Harper, 2006. 1-341. Print.



### EXTENSIONS:

If using the film after completing the other lessons in this unit, offer the students a prompt to consider the film vs the information they have learned about Pompe disease and the individuals involved, to compare and contrast actual events to the Hollywood version. They can write their thoughts and/or share them aloud, engaging the students in a whole class discussion.



# Student Video: “Extraordinary Measures”

NAME \_\_\_\_\_

DATE \_\_\_\_\_

*Instructions: Answer questions # 1-14 while viewing the film.*

1. Megan and Patrick Crowley suffer from \_\_\_\_\_ Disease.
2. This disease causes \_\_\_\_\_ deterioration in all regions of the body.
3. List at least two symptoms common in individuals suffering from this disease.
  - a. \_\_\_\_\_
  - b. \_\_\_\_\_
4. The disease is a \_\_\_\_\_ disorder. Individuals suffering are missing an \_\_\_\_\_ which metabolizes sugar (glycogen).
5. Briefly explain what happens to the excess sugar (glycogen) in the bodies of individuals suffering from the disease?
6. Many scientists have attempted to create and inject sufferers of this disease with enzymes. Unfortunately, this method of treatment did not work. Why not?
7. Briefly explain how the enzyme Dr. Stonehill was attempting to create differed from those that were previously manufactured.
8. The largest obstacle Dr. Stonehill's encountered in conducting his research was obtaining adequate \_\_\_\_\_.
9. Briefly explain why it was decided that the clinical trial for the drug created for Pompe Disease would only be initially administered to infants.

**STUDENT VIDEO: "EXTRAORDINARY MEASURES"** (continued...)

10. TRUE or FALSE: Dr. Stonehill's enzyme was the one chosen by the pharmaceutical corporation for pharmaceutical trials.
11. What is a sibling trial?
12. Shortly after Megan and Patrick were injected with the trial medicine, they began laughing. Explain what this indicated and why they were laughing?
13. Currently, if the medicine for Pompe Disease is administered to infants, it can control the \_\_\_\_\_ of the disease for life.
14. TRUE or FALSE: Recent laboratory results have shown that Dr. Stonehill's visionary scientific theory was correct.

*Instructions:* Answer questions # 15-17 after viewing the film.

15. The film portrays corporate pharmaceutical companies to be solely concerned with profits rather than human health. How could this corporate mindset impact you as a consumer of pharmaceutical products?
16. The notion that researchers should always make scientific decisions objectively was a recurring theme throughout the film? Why must scientists make decisions objectively? Do you think it is reasonable for exceptions to be made? Explain why or why not.
17. During the film Mr. Crowley noted that many of the scientists working to find a cure for Pompe disease had never even seen a person suffering from the disease. Do you think biomedical researchers should come into contact with individuals suffering from the disease they are studying? Explain why or why not.



## Student Video: “Extraordinary Measures”

1. (6:45) Megan and Patrick Crowley suffer from Pompe Disease.
2. (12:50) This disease causes muscle deterioration in all regions of the body.
3. (13:30) List at least two symptoms common in individuals suffering from this disease.  
Respiratory complications, Enlarged organs (heart, liver, etc.), muscle weakness
4. (20:55) The disease is a genetic disorder. Individuals suffering from this disease are missing an enzyme which metabolizes sugar (glycogen).
5. (21:00) Briefly explain what happens to the excess sugar in the bodies of individuals suffering from the disease?  
The excess sugar (glycogen) is absorbed by the lysosomes but cannot be broken down. The lysosomes swell, interfering with normal muscle cells and eventually burst and causes deterioration of surrounding cells.
6. (21:20) Many scientists have attempted to create and inject sufferers of this disease with enzymes. Unfortunately, this method of treatment did not work. Why not? Their cells would not take in the enzymes that were being injected in them.
7. (21:33) Briefly explain how the enzyme Dr. Stonehill is attempting to create differs from those that were previously manufactured. The enzyme he is creating has a biological marker that enables the cell to take in the enzyme.
8. (22:30) The largest obstacle Dr. Stonehill encountered in conducting his research was obtaining adequate funding.
9. (1:22:15) Briefly explain why it was decided that the clinical trial of the drug created for Pompe Disease would only be initially administered to infants. It was decided the clinical trials of medicine for Pompe Disease would only be administered to infants because they require less of the medicine (enzyme), the initial supply was limited, and it had a much greater potential for working in infants
10. (1:20:05) TRUE or FALSE: Dr. Stonehill’s enzyme was the one chosen by the pharmaceutical corporation for pharmaceutical trials.
11. (1:25:25) What is a sibling trial? A sibling trial is an experiment or trial that is conducted on just two patients that have the same disease and genetic inheritance (siblings).
12. (1:39:47) Shortly after Megan and Patrick were injected with the trial medicine, they began laughing. Explain what this indicated and why they were laughing? Megan and Patrick’s laughter indicated that the medicine was working. The children had a sugar high. Thus, the enzyme was effectively breaking down the sugar in their body.
13. (1:41:26) Now, if an infant is diagnosed with Pompe Disease they can be given a special medicine that controls the symptoms the disease for life.
14. (1:41:41) TRUE or FALSE: Recent laboratory results have shown that Dr. Stonehill’s visionary scientific theory was correct.
15. The film portrays corporate pharmaceutical companies to be solely concerned with profits rather than human health. How could this corporate mindset impact you as a consumer of pharmaceutical products? Answers will vary.
16. The notion that researchers should always make scientific decisions objectively was a recurring theme throughout the film? Why must scientists make decisions objectively? Do you think it is reasonable for exceptions to be made? Explain why or why not. Answers will vary.
17. During the film Mr. Crowley noted that many of the scientists working to find a cure for Pompe Disease had never even seen a person suffering from the disease. Do you think biomedical researchers should come into contact with individuals suffering from the disease they are studying? Explain why or why not. Answers will vary.

# Resources and References



## Resources:

Description	Source	Catalog #	Price
Phenol red indicating solution 0.2% aqueous solution (100mL)	Fisher Scientific <a href="http://www.fishersci.com">www.fishersci.com</a>	S71434	\$5.85
pH 3.0 buffer solution	Fisher Scientific <a href="http://www.fishersci.com">www.fishersci.com</a>	S06487	\$8.25
pH 10.0 buffer solution	Fisher Scientific <a href="http://www.fishersci.com">www.fishersci.com</a>	S06494	\$8.25
Filter paper Paper, 8cm x 10.5cm; 100 sheets	Fisher Scientific <a href="http://www.fishersci.com">www.fishersci.com</a>	88600	Pk of 100, \$49.98
Disposable transfer pipets (3mL total volume)	Fisher Scientific <a href="http://www.fishersci.com">www.fishersci.com</a>	13-711-7M	Pk of 500, \$49.60
Microfuge tubes (1.5mL), assorted colors, nonsterile	Fisher Scientific <a href="http://www.fishersci.com">www.fishersci.com</a>	02-682-556	Pk of 500, \$33.64
Sample collection cups	Science Take-Out <a href="http://www.sciencetakeout.com">www.sciencetakeout.com</a>	SC15	Pk of 50, \$5.00
From DNA to Protein Structure and Function	Science Take-Out <a href="http://www.sciencetakeout.com">www.sciencetakeout.com</a>	STO-106 (individual assembled kit) STO-106U (unassembled classroom sets)	1 assembled kit = \$8 1 pk of 10 unassembled kits = \$38 (bulk discounts available)

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Ask the Experts: What Is Pompe Disease? Scientific American brief article (not the best) <http://www.scientificamerican.com/article.cfm?id=pompe-disease>

Beesley, E., A.H. Child, M.H. Yacoub, The Identification of Five Novel Mutations in the Lysosomal Acid-(1,4) Glucosidase Gene from Patients with Glycogen Storage Disease Type II, *Mutations in Brief*, Brief #134 (1997).

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- From the research group in the Netherlands, this paper presents the results of a one-center study to evaluate the response in older children (5-18 years of age) to ERT with the commercial product Myozyme. The sample size is quite small, only 5 individuals, but the results were favorable. Unlike the trial in infants, there were no infusion adverse reactions and all patients showed levels of improvement. Additionally, this study presents genotype analysis, as do all studies from the Netherlands group. Fairly easy to read and understand with excellent tables summarizing data and results. **Myozyme clinical trial.** [http://amdapompe.ehclients.com/downloads/publications/MiniLOTS\\_Genzyme\\_Sponsored\\_Single\\_Center\\_Study.pdf](http://amdapompe.ehclients.com/downloads/publications/MiniLOTS_Genzyme_Sponsored_Single_Center_Study.pdf)
- Van Capelle, C.I., L.P.F. Winkel, M.L.C. Hagemans, S.K. Shapira, W.F.M. Arts, P.A. van Doorn, W.C.J. Hop, A.J.J. Reuser, A.T. van der Ploeg, Eight years experience with enzyme replacement therapy in two children and one adult with Pompe disease, *Neuromuscular Disorders*, **18**:447-452, (2008).
- Excellent follow-up study of three individuals who started enzyme replacement therapy with transgenic GAA produced in rabbit milk in 1999. This report illustrates that transgenic animals can be used successfully to produce therapies and the individuals in this small trial benefited. Briefly mentioned in this paper is the transition of the patients from rabbit GAA to CHO GAA. Well written, easy to read. The Methods and Results should be read as well. **Rabbit ERT.**
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- It provides a very nice overview of the ERT research that has taken place using transgenic milk as well as the now commercial product produced in CHO cells. The editorial highlights successes as well as lessons learned and further questions that still need to be answered. **Enzyme Replacement Therapy**
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# Content Assessment

NAME \_\_\_\_\_

DATE \_\_\_\_\_

Circle One: Pre-test

Post-test

**Part I. True-False:** Write True or False in the blank next to each statement.

- \_\_\_\_\_ An enzyme is a protein.
- \_\_\_\_\_ A recessive disease is inherited from both parents.
- \_\_\_\_\_ Pathology can be attributed to changes in genotype.
- \_\_\_\_\_ Enzyme replacement therapy has been used to treat many disorders.
- \_\_\_\_\_ Gene therapy treatment undergoes the same scrutiny as other pharmaceutical products.

**Part II. Multiple Choice:** Write the letter of the correct answer in the blank next to each item.

- \_\_\_\_\_ 1. Pompe disease is a type of:  
A. Blood disease      B. Cancer      C. Lysosomal storage disease      D. Sex-linked disease
- \_\_\_\_\_ 2. Glycogen is commonly found in abundance in  
A. Mitochondria      B. Muscle tissue      C. Nucleus      D. Plant cells
- \_\_\_\_\_ 3. Gene therapy makes use of  
A. Bacteria      B. Plants      C. Protozoa      D. Virus
- \_\_\_\_\_ 4. The development of Myozyme to treat Pompe disease was the result of  
A. A large pharmaceutical company      B. Many individuals      C. One father's crusade      D. One researcher's work
- \_\_\_\_\_ 5. Individuals with Pompe disease lack sufficient quantities of which enzyme  
A. Acid alpha glucosidase      B. Adenosine triphosphate      C. Carbonic anhydrase      D. Superoxide dismutase

**Part III. Short answer:** Write your answers in the spaces below each item.

1. Transcribe the following sequence:  
TAC AAA GAA TAA TGC ATA ACA
2. Briefly describe the clinical differences between early and late on-set Pompe disease.





# Content Assessment

Circle One: Pre-test

Post-test

**Part I. True-False:** Write True or False in the blank next to each statement.

- True An enzyme is a protein.
- True A recessive disease is inherited from both parents.
- True Pathology can be attributed to changes in genotype.
- False Enzyme replacement therapy has been used to treat many disorders.
- False Gene therapy treatment undergoes the same scrutiny as other pharmaceutical products.

**Part II. Multiple Choice:** Write the letter of the correct answer in the blank next to each item.

- C 1. Pompe disease is a type of:  
A. Blood disease      B. Cancer      C. Lysosomal storage disease      D. Sex-linked disease
- B 2. Glycogen is commonly found in abundance in  
A. Mitochondria      B. Muscle tissue      C. Nucleus      D. Plant cells
- D 3. Gene therapy makes use of  
A. Bacteria      B. Plants      C. Protozoa      D. Virus
- B 4. The development of Myozyme to treat Pompe disease was the result of  
A. A large pharmaceutical company      B. Many individuals      C. One father's crusade      D. One researcher's work
- A 5. Individuals with Pompe disease lack sufficient quantities of which enzyme  
A. Acid alpha glucosidase      B. Adenosine triphosphate      C. Carbonic anhydrase      D. Superoxide dismutase

**Part III. Short answer:** Write your answers in the spaces below each item.

- Transcribe the following sequence:  
TAC AAA GAA TAA TGC ATA ACA  
mRNA= AUG UUU CUU AUU ACG UAU UGU
- Briefly describe the clinical differences between early and late on-set Pompe disease.  
Early on-set is the classical infantile form. Symptoms are usually evident in the first few months of birth and include an enlarged heart, respiratory difficulties, and weak muscles causing difficulty eating, swallowing, sitting, and holding head up  
  
Late on-set usually does not have cardiac involvement and is more confined to limb-girdle muscles. Patients often have difficulty with walking and motor skills and can also develop respiratory difficulties.



# Content Area Expert Evaluation (page 1)

Thank you for reviewing *The Pompe Predicament* curriculum. Please review the entire curriculum and then complete the questions below. You are welcome to insert comments directly in the manual as well as in the section provided below. Comments and suggestions are greatly appreciated!

REVIEWER NAME:

DATE REVIEWED:

EMPLOYER:

DEPARTMENT/DIVISION:

JOB TITLE:

EMAIL:

**Part I:** For each item below, please indicate your response to each question as it relates to the curriculum **overall** by circling Yes (Y), No (N), or Undecided (U).

1. Is the science content in the curriculum accurate?	Y	N	U
2. Is the science content in the curriculum current?	Y	N	U
3. Is the science content in the curriculum important for science literacy?	Y	N	U
4. Is the content in the curriculum related to major biological concepts? (e.g., molecular genetics)	Y	N	U
5. Is the content coverage in the curriculum thorough and complete?	Y	N	U
6. Are potential misconceptions adequately addressed?	Y	N	U
7. Is the content in the curriculum properly sequenced for a novice?	Y	N	U
8. Are there additional concepts that should be included? (If yes, please elaborate below.)	Y	N	U

**Part II:** Please include below any comments or suggestions about the curriculum.

1. GENERAL COMMENTS ABOUT THE OVERALL CURRICULUM:

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# Content Area Expert Evaluation (page 2)

**Part II (continued):** Please include below any comments or suggestions about the curriculum.

## 2. COMMENTS REGARDING INDIVIDUAL LESSONS:

<p><b>LESSON 1:</b> Through a Father's Eyes</p>	
<p><b>LESSON 2:</b> Pompe Timeline</p>	
<p><b>LESSON 3:</b> Journal Club</p>	
<p><b>LESSON 4:</b> DNA → Protein</p>	
<p><b>LESSON 5:</b> Putting It All Together</p>	
<p><b>LESSON 6:</b> Grand Rounds</p>	
<p><b>LESSON 7:</b> Gene Therapy Role Play</p>	
<p><b>LESSON 8:</b> Movie Extraordinary Measures</p>	



# Teacher Feedback Form (page 1)

Thank you for reviewing *The Pompe Predicament* curriculum. Please review the entire curriculum and then complete the questions below. You are welcome to insert comments directly in the manual as well as in the section provided below. Comments and suggestions are greatly appreciated!

TEACHER NAME:

DATE REVIEWED:

SUBJECTS TAUGHT:

GRADE LEVEL TAUGHT:

SCHOOL:

EMAIL:

## Part I: Evaluation of the entire curriculum:

**SECTION A:** For each item below, please indicate your response to each question as it relates to the curriculum **overall** by marking Strongly Agree (SA), Agree (A), Undecided (U), Disagree (D), or Strongly Disagree (SD).

	SA	A	U	D	SD
1. Are the experimental procedures appropriate for your students?					
2. Are the topics addressed important for your course objectives?					
3. Are the topics addressed relevant to your students' lives?					
4. Are the topics addressed interesting to your students?					
5. Is the depth of coverage of topics appropriate?					
6. Is the overall quality of the curriculum satisfactory?					
7. Is the content in the curriculum properly sequenced?					
8. Is the content in the curriculum adaptable for a range of student ability levels?					

**Part II:** Please provide additional comments pertaining to the laboratory manual overall.

**SECTION B:** Please provide additional comments pertaining to the laboratory manual **overall**.

1. Are there any topics/sections that should be added to/deleted from the curriculum? If so, please explain.

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2. Additional comments

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# Teacher Feedback Form (page 2)

## Part II: Evaluation of individual lessons

SECTION A: For each question below, please indicate your response for each **specific lesson** by marking High, Moderate, Low, or Not Applicable (NA).

	Lesson 1: Looking Through a Father's Eyes				Lesson 2: Pompe Timeline				Lesson 3: Journal Club				Lesson 4: DNA → Protein			
	High	Mod- erate	Low	NA	High	Mod- erate	Low	NA	High	Mod- erate	Low	NA	High	Mod- erate	Low	NA
1. Is the amount of background information sufficient?																
2. Do you feel you were provided adequate advance instruction?																
3. Were you provided enough time to complete the lesson?																
4. Is the procedure clearly written?																
5. Is the suggested assessment sufficient?																

	Lesson 5: Putting it All Together				Lesson 6: Grand Rounds				Lesson 7: Gene Therapy Role Play				Lesson 8: Extraordinary Measures – Movie			
	High	Mod- erate	Low	NA	High	Mod- erate	Low	NA	High	Mod- erate	Low	NA	High	Mod- erate	Low	NA
1. Is the amount of background information sufficient?																
2. Do you feel you were provided adequate advance instruction?																
3. Were you provided enough time to complete the lesson?																
4. Is the procedure clearly written?																
5. Is the suggested assessment sufficient?																



# Teacher Feedback Form (page 3)

**Part II (continued):**

SECTION B: Please provide additional comments pertaining to each specific lesson.

Lesson	Are there any topics, sections, or resources that should be added or deleted? If so, please explain.	Additional Comments
LESSON 1: Through a Father's Eyes		
LESSON 2: Pompe Timeline		
LESSON 3: Journal Club		
LESSON 4: DNA → Protein		
LESSON 5: Putting It All Together		
LESSON 6: Grand Rounds		
LESSON 7: Gene Therapy Role Play		
LESSON 8: Movie Extraordinary Measures		



# Student Feedback Form (page 1)

STUDENT NAME:		DATE REVIEWED:	
STUDENT GRADE LEVEL:		SCHOOL NAME:	
SCHOOL:	TEACHER:	SUBJECT:	

**Section A: Evaluation of individual lessons** For each question below, please indicate your response for each **specific lesson** by marking High, Moderate, Low, or Not Applicable (NA).

	Lesson 1: Looking Through a Father's Eyes				Lesson 2: Pompe Timeline				Lesson 3: Journal Club				Lesson 4: DNA → Protein			
	High	Mod- erate	Low	NA	High	Mod- erate	Low	NA	High	Mod- erate	Low	NA	High	Mod- erate	Low	NA
1. Is the amount of background information sufficient?																
2. Do you feel you were provided adequate advance instruction?																
3. Were you provided enough time to complete the lesson?																
4. Is the procedure clearly written?																
5. Is the suggested assessment sufficient?																
	Lesson 5: Putting it All Together				Lesson 6: Grand Rounds				Lesson 7: Gene Therapy Role Play				Lesson 8: Extraordinary Measures – Movie			
1. Is the amount of background information sufficient?																
2. Do you feel you were provided adequate advance instruction?																
3. Were you provided enough time to complete the lesson?																
4. Is the procedure clearly written?																
5. Is the suggested assessment sufficient?																



# Student Feedback Form (page 2)

**Section B:** Please provide additional comments pertaining to each specific lesson.

Lesson	Are there any topics, sections, or resources that should be added or deleted? If so, please explain.	Additional Comments
LESSON 1: Through a Father's Eyes		
LESSON 2: Pompe Timeline		
LESSON 3: Journal Club		
LESSON 4: DNA → Protein		
LESSON 5: Putting It All Together		
LESSON 6: Grand Rounds		
LESSON 7: Gene Therapy Role Play		
LESSON 8: Movie Extraordinary Measures		







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