NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

A Ticket to Nowhere:
Lysosomal Storage Disorders

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Preliminary – The Path of a Lysosomal Hydrolase

In medical school, Rebecca Crumpler was taught the specific mechanisms of lysosomal hydrolase protein trafficking. She knew that proteins must arrive at the correct destination in order to function properly. She thought of different proteins like pieces of luggage that need to get to different parts of the country. Each piece of luggage needs to receive the appropriate ticket at the airport to ensure that it arrives at the correct location.

You are now a student intern under the mentorship of Dr. Crumpler. She advises you to watch the following video on lysosomal protein trafficking:

- *Golgi: Protein Modification*. Running time: 3:50 min. Produced by WWWIC at NDSU, Virtual Cell Animation Collection, 2008. <https://youtu.be/u38LjCOvDZU>

Tasks

While you watch:

- Illustrate the cross-section of a cell to visualize the path taken by a lysosomal hydrolase from the endoplasmic reticulum (ER) to the lysosome.
- Include and label the ER, the hydrolase protein, the core oligosaccharide, the mannose-6-phosphate (M6P), the M6P receptor, transport vesicles, the endosome, and the lysosome.
- Indicate with directional arrows the important sequential steps necessary for proper trafficking of lysosomal hydrolases to the lysosome.

Part I – Meet the Silva Family

Maria and Francisco Silva were so happy with the arrival of their new baby girl, Ana Sophia. Ana's first months of life were uneventful, consisting of a lot of eating and sleeping. By the time she was six months old, however, her parents had some concerns about her growth and development. Ana hadn't gained weight in the past month and had noticeable clubfeet. She was unable to hold up her head or roll over.

At her six-month wellness visit, Ana's parents expressed their concerns to their pediatrician, Dr. Rebecca Crumpler, who validated their fears and shared some further concerns with the new parents. In addition to Ana's "failure to thrive" and clubfeet, Dr. Crumpler noticed some abnormal facial features as well as significantly reduced mobility of her hips and knees. Dr. Crumpler told Maria and Francisco that to determine what was going on she would run some laboratory tests.

When Dr. Crumpler got back to her office, she started some preliminary research on Ana's case. In medical school, Dr. Crumpler had learned about a set of rare disorders called lysosomal storage disorders (LSDs) that are characterized by an accumulation of undigested substrates in the lysosomes. Accumulation of the undigested substrates results typically from a lack of functional lysosomal hydrolases, proteins that are targeted to, move into, and function within the lysosome to degrade waste. As a cell ages and more undigested substrates build-up, the formation of large vesicles occurs within a variety of cell types including chondrocytes, which are cells that help form cartilage.

Dr. Crumpler has invited you as the student intern to help with her research. She asks you to investigate the role of chondrocytes in cartilage development and the effect of LSDs on this process. She explains to you that she suspects that some of Ana Sophia's signs and symptoms, particularly her reduced mobility and abnormal facial features, may be due to abnormal cartilage development, and that cartilage plays an important role throughout the body including joints between bones, facial features such as the external ear and nasal septum, and other structures including the trachea (windpipe).

Your research leads you to find that chondrocytes are the only cell type resident in cartilage tissue and that they are responsible for the synthesis and maintenance of the extracellular cartilage matrix that makes up the cartilage tissue. You find the images shown in Figure 1 below that compare cartilage tissue in a healthy patient (left image) to that found in an LSD patient (right image). You are excited to share with Dr. Crumpler that the cartilage matrix produced by chondrocytes (in purple) is less dense in patients with an LSD and that the chondrocytes from such patients are enlarged with vesicles filled with undigested substrates; however, you do not yet understand how these two phenotypes are related.

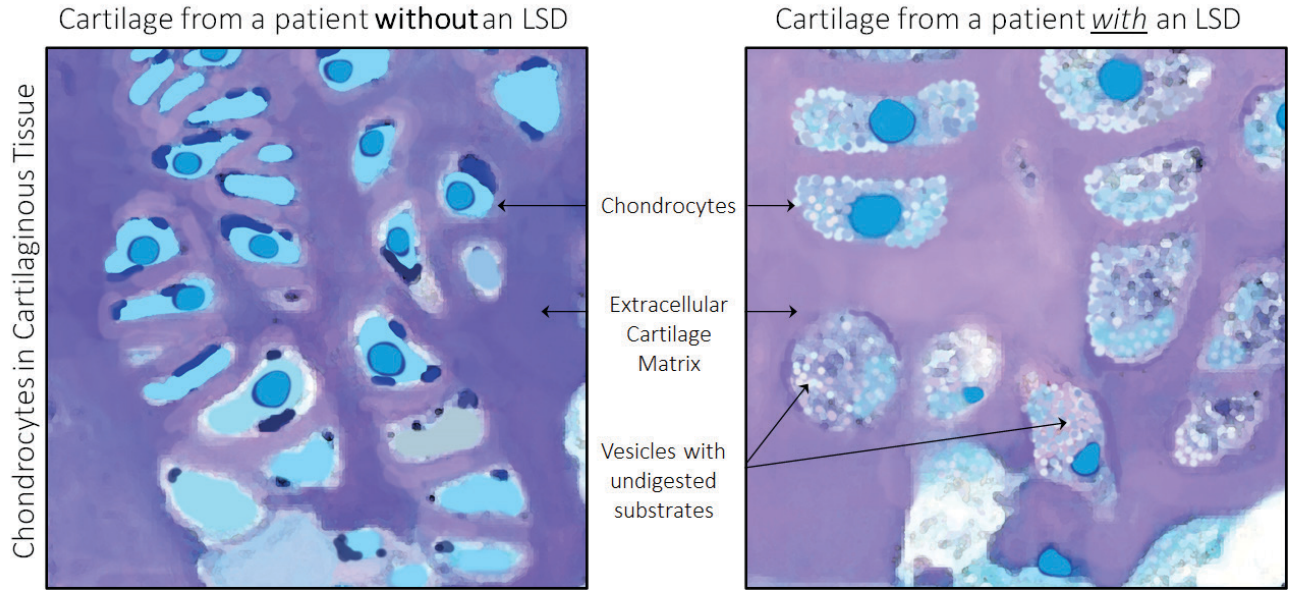

Figure 1. Illustration of chondrocytes in touline-blue stained cartilaginous tissue. *Left:* normal chondrocytes (nuclei are dark blue, cytoplasm is light blue) with normal production and secretion of extracellular cartilaginous matrix (purple). *Right:* larger chondrocytes with enlarged vesicles containing undigested substrates (punctate intracellular areas that appear white) with reduced extracellular cartilaginous matrix (light purple and pink) are features indicative of LSDs. *Image credit:* Tracy Rosebrock and Rachel Hirst.

Dr. Crumpler explains that your research is helping her to narrow down her diagnosis for Ana. She shares with you that there are over 50 different types of LSDs and that these disorders are genetically encoded and inherited. The symptoms of each type of LSD vary, depending on a variety of factors including the underlying genetic mutation, the resulting loss or impaired activity of the encoded protein, the effect on lysosome functioning, the type of substrates that accumulate in the lysosome, and the cell type most affected.

Dr. Crumpler explains Gaucher's disease, the most common type of LSD, using Figure 2. Gaucher's disease is caused by a mutation in the GBA (beta-glucosidase) gene, which normally encodes the lysosomal enzyme glucocerebrosidase. In healthy patients, glucocerebrosidase catalyzes the hydrolysis of glucocerebroside to the metabolites, glucose and ceramide. Those with Gaucher's disease have a deficiency in glucocerebrosidase.

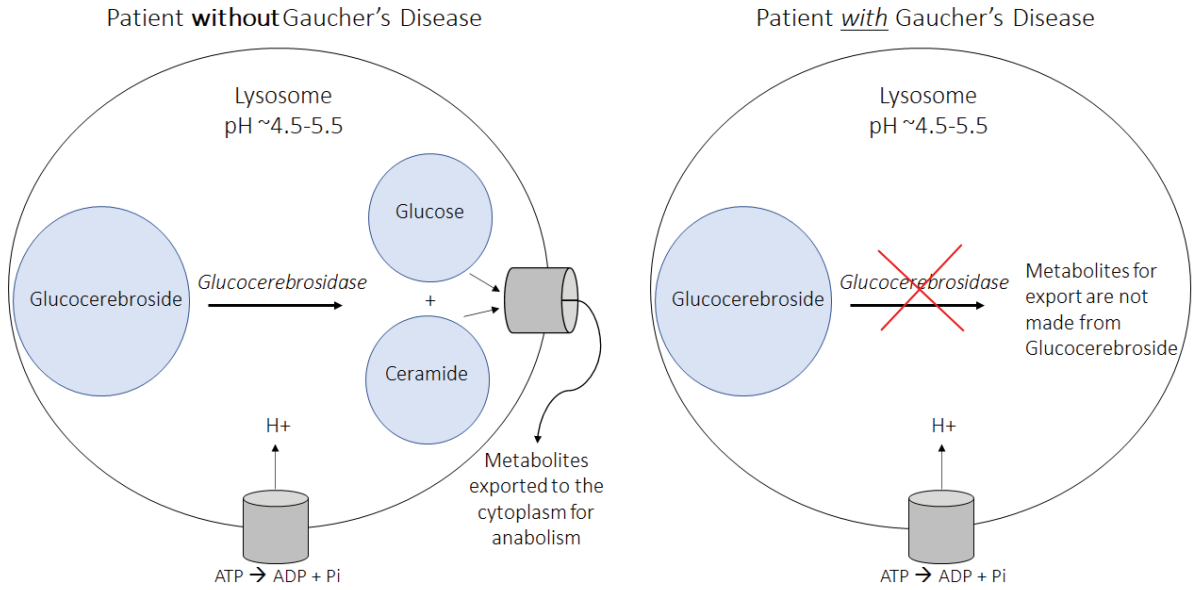

Figure 2. Illustration of glucocerebroside catabolism in the lysosome in a patient without Gaucher's disease (left) compared to a patient with Gaucher's disease (right). Glucocerebroside is catabolized to the metabolites glucose and ceramide by the lysosomal hydrolase, glucocerebrosidase. Glucose and ceramide are exported to the cytoplasm for anabolism by the cell. In those with Gaucher's disease, glucocerebrosidase is not present or partially inactive, which stops or slows the catabolism of glucocerebroside. *Image credit:* Tracy Rosebrock and Rachel Hirst.

Ana's signs and symptoms are not consistent with Gaucher's disease. Dr. Crumpler suspects a few other types of LSDs, one of which is more common in people of Portuguese ancestry, like the Silva family. Together, you have collected important clues but not enough to diagnose Ana Sophia. You and Dr. Crumpler still have a lot of work to do.

Questions

1. Compare the phenotype of chondrocytes from a patient without an LSD to a patient with an LSD as shown in Figure 1. What is the main function of the lysosome based on these results?

2. Based on what you know about the function of lysosomes within cells, the function of chondrocytes in cartilage tissue, and the results from Figure 1, come up with a hypothesis for why lysosomal dysfunction could result in Ana's reduced hip and knee mobility.

3. In those with Gaucher's disease, which substrate would accumulate in its undigested form in the lysosomal vesicle?

4. In Gaucher's disease, one enzyme is deficient in the lysosome. What would be the expected result if multiple lysosomal hydrolases failed to travel to or stay within the lysosome?

Part II – The Test Results

Dr. Crumpler ordered a panel of tests to figure out which of Ana's lysosomal hydrolases might be causing her symptoms. She knew it was also important to determine the location of the hydrolases. Was it inside the cell within the lysosome (intracellular lysosomal)? Or outside of the cell (extracellular)?

To look for intracellular lysosomal hydrolases, Dr. Crumpler sampled Ana's skin cells (fibroblasts) and had them cultured in the lab. The fibroblast cells and intracellular organelles were lysed (broken open) to retrieve any intracellular lysosomal hydrolases. Cell lysates containing any intracellular hydrolases were then mixed with substrates that matched each individual lysosomal hydrolase enzyme. The solution was buffered to the correct acidic pH to promote the enzymatic activity of the hydrolases (Figure 3, next page). Dr. Crumpler also took a sample of Ana's blood to look for lysosomal hydrolases that may have been mis-localized and instead secreted into the extracellular space, in this case the blood. The lab buffered the blood sera to the correct acidic pH and added the matching substrates for individual hydrolase enzymes (Figure 4, next page). Both tests used hydrolase activity as a marker for the presence of the hydrolase.

Dr. Crumpler invites you to the upcoming weekly clinical case discussion group where Ana's case and results will be discussed. She shares Ana's lab results with you (Figures 3 and 4) before the meeting and asks you to be ready to join the group's conversation. During the meeting the following questions were asked. What do you think?

Questions

1. Why did the lab assays require buffering both the cell lysates and the blood sera to an acidic pH to test for enzymatic activity of the lysosomal hydrolases?

2. Which of Ana's lysosomal hydrolases had lower activity levels in the buffered fibroblast cell lysate compared to the healthy control patient (see Figure 3)?

3. Which of Ana's lysosomal hydrolases had higher activity levels in the buffered blood sera compared to the healthy control patient (see Figure 4)?

4. Compare the results obtained in the fibroblast extract assay (Figure 3) to the blood assay (Figure 4). Which of the following two hypotheses is more likely to be true?
   - *Hypothesis 1:* Ana's lysosomal hydrolases are present intracellularly (inside the fibroblasts) but lack enzymatic activity, even in buffered acidic conditions.
   - *Hypothesis 2:* Ana's lysosomal hydrolases can function as enzymes in acidic conditions but have been secreted to the extracellular space.

5. How can the presence of multiple lysosomal hydrolases in extracellular fluid (like blood) rather than intracellularly within the lysosome, be explained using cellular biology (review the preliminary section of this case, "The Path of a Lysosomal Hydrolase")? Is it more likely that Ana has a mutation in each gene that encodes a lysosomal hydrolase, or that there is a mistake happening in a process shared by the hydrolases?

6. Given what you have learned from these data and the information in Part I, what is happening in Ana's chondrocyte cells to cause the phenotype observed in cartilaginous tissue?

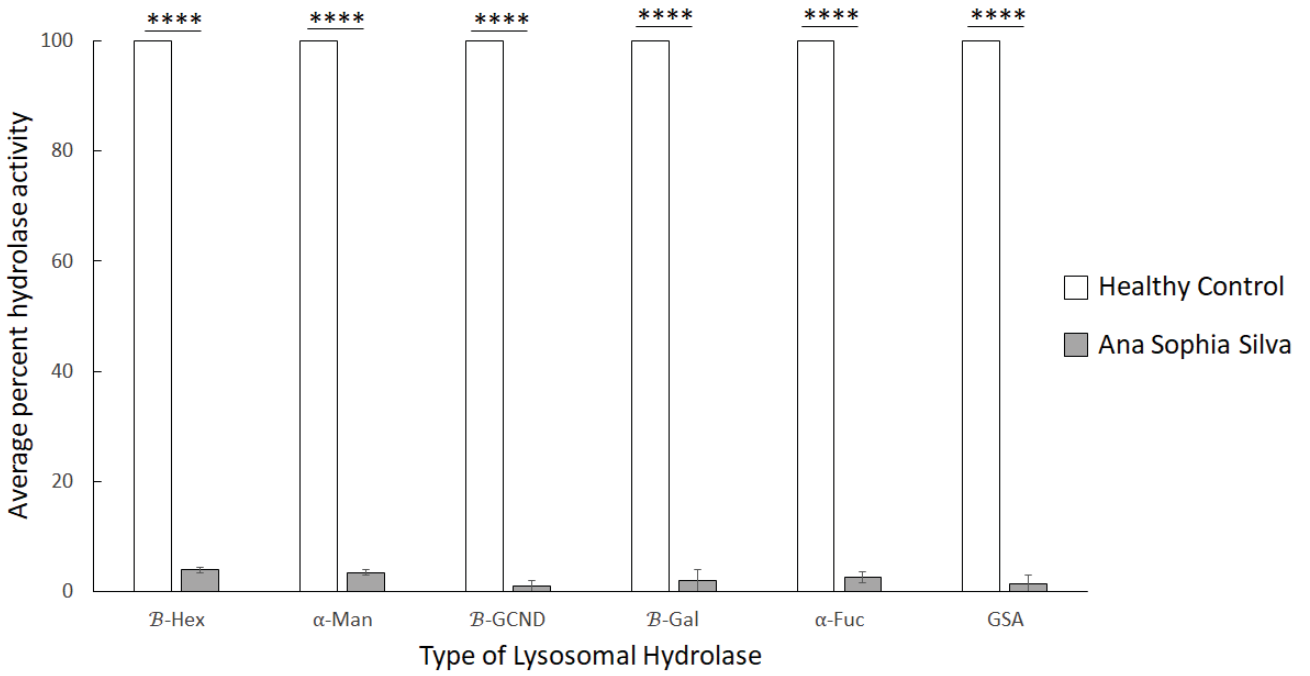

Figure 3. Quantification of Ana's intracellular lysosomal hydrolase activity. The figure shows the average percent lysosomal hydrolase activity in Ana's fibroblasts compared to a healthy control patient. Error bars equal standard deviation. β-Hex = β-hexosaminidase, α-Man = α–mannosidase, β-GCND = β-glucuronidase, β-Gal = β-galactosidase, α-Fuc = α-fucosidase, GSA = Glycosylasparaginase. **** = p<0.0001; ***=p<0.001.

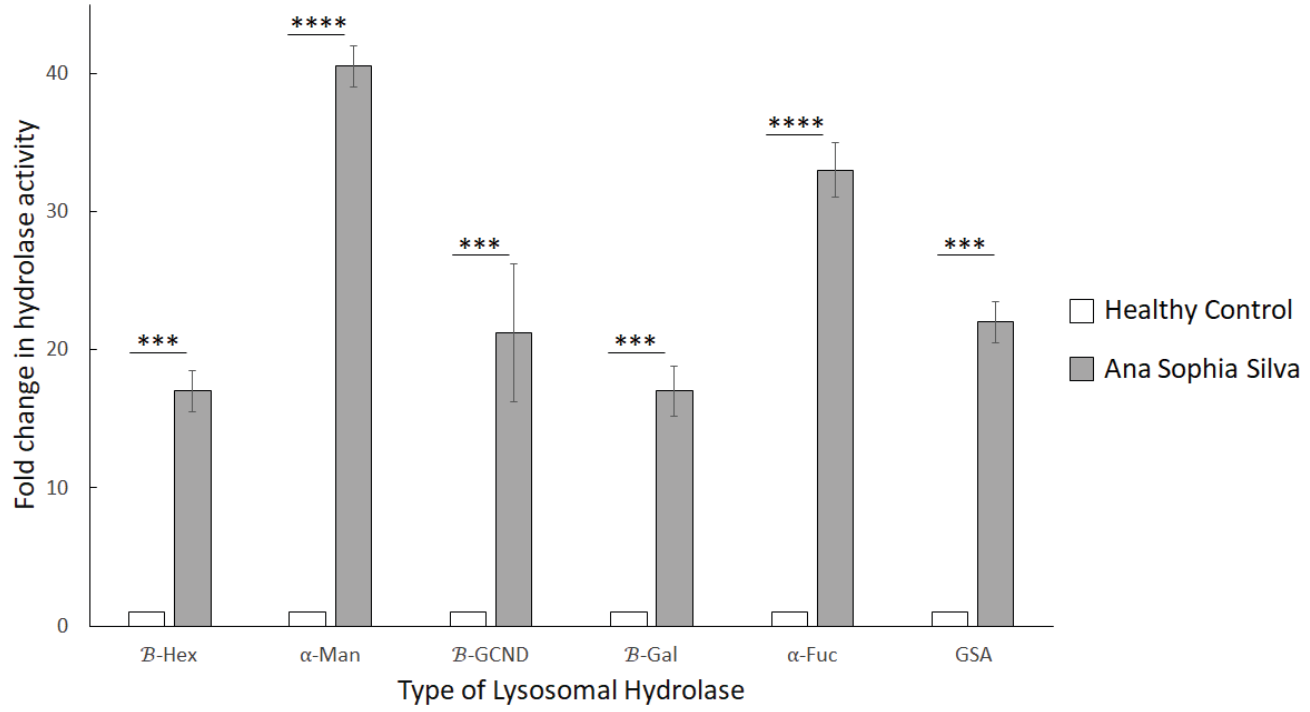

Figure 4. Quantification of Ana's extracellular lysosomal hydrolase activity. The figure shows the average fold change of lysosomal hydrolase activity in Ana's blood compared to a healthy control patient. Error bars equal standard deviation. β-Hex = β-hexosaminidase, α-Man = α–mannosidase, β-GCND = β-glucuronidase, β-Gal = β-galactosidase, α-Fuc = α-fucosidase, GSA = Glycosylasparaginase. **** = p<0.0001; ***=p<0.001.

Part III – Putting It All Together

Ana's test results show that multiple lysosomal hydrolases are affected in the same way by Ana's condition. In preparation for her next meeting with the Silva family, Dr. Crumpler refreshes her memory on lysosomal protein trafficking. You do too! You review your notes on Dr. Crumpler's analogy relating intracellular protein trafficking to the labeling, sorting, and delivery of luggage by airlines and remember that Ana's fibroblast and blood assays revealed that multiple lysosomal hydrolases were mis-localized. You suspect that the hydrolases were not "tagged" correctly. Dr. Crumpler asks you to think through the pathway of lysosomal protein trafficking to identify many possible explanations for Ana's condition.

Use the pathway you drew at the beginning of this case study to help answer the questions below.

Questions

1. Identify at least four places along the lysosomal protein's pathway that if disrupted would result in improper lysosomal function.

2. Write these possible pathway disruptions in the first column of the table below and fill in the rest of the chart including whether the hydrolase would be functional and if it would be in the lysosome or the extracellular space (blood sera).

Possible Pathway Disruption	*Functional Hydrolase? (Yes or No)*	*Hydrolase in the Lysosome? (Yes or No)*	*Hydrolase in the Extracellular Space (Blood sera)? (Yes or No)*

3. Given your hypothetical pathway disruptions, which would result in lysosomal hydrolases being sorted incorrectly and secreted extracellularly into Ana's blood?

4. What would be a logical next test Dr. Crumpler could order to provide the family with a more definitive diagnosis given the greater than 50 known LSDs and their underlying causes?

Follow-Up Assignment

Dr. Crumpler invites you to her office. She has just received the genetic test results for Ana Sophia that she ordered with consent from the Silva family. You hold your breath watching Dr. Crumpler open and begin reading the results. You know that the prognosis for LSDs varies depending on the type of genetic mutation causing the specific LSD. You are both worried because the LSD Dr. Crumpler most suspects is severe. The results are shown below.

> ANA SOPHIA SILVA — GENETIC TESTING RESULTS
>
> *Gene:* T to A substitution at nucleotide 2601 in *GNPTAB* gene encoding N-acetylglucosamine-1-phosphate transferase alpha and beta subunits.
>
> *Protein*: Change from a tyrosine to a premature stop codon at position 867 of the protein sequence (Y867X).

Unfortunately, Dr. Crumpler's preliminary diagnosis for Ana is correct. She needs to prepare to talk with the Silva family. Dr. Crumpler asks you to gather important details for her discussion with Ana's parents. She wants to be sure to give them enough details so they can understand the disease, but also be compassionate about Ana's diagnosis.

Using the information that you learned today, and further research done as homework, draft a one-page description of Ana's disease for Dr. Crumpler to use when she meets with the Silva family to tell them about Ana's diagnosis. Be sure to explain the following for a non-scientist audience (Ana's parents):

1. What an LSD is in general.
2. Ana's specific LSD (the recommended websites below and the genetic test results above will lead you to this answer).
3. The gene affected and the normal function of its protein product.
4. How the path of multiple lysosomal hydrolases is altered because of this gene mutation.
5. How Ana's LSD changes chondrocyte biology resulting in Ana's signs and symptoms.
6. Ana's overall prognosis.

Recommended Websites

- MedlinePlus. *GNPTAB* gene: N-acetylglucosamine-1-phosphate transferase subunits alpha and beta. <https://medlineplus.gov/genetics/gene/gnptab/>
- National Organization for Rare Disorders (NORD). Rare disease database: I-cell disease. <https://rarediseases.org/rare-diseases/i-cell-disease/>