I-cell Disease (Mucolipidosis II): A Rare Lysosomal Storage Disorder

Background and Introduction

Framed in the living room at my cousin's house in Philadelphia is a picture of a young boy. He is dressed in bright red and is smiling from ear to ear. I have seen this image countless times in photo albums, family group chat messages, and on social media. In other pictures, he is watching *Sesame Street,* playing with his brother, Kavi, and bonding with friendly nurses. According to these pictures, he seemed to do everything with a smile on his face. My first cousin, Yash Gandhi, was born on December 23, 2000 to Sonal and Ashesh Gandhi. At 11 months, Yash was diagnosed with a terminal rare illness called I-cell disease also known as mucolipidosis II (MLII). At the time, information regarding MLII was scarce. My aunt and uncle recount the day Yash received his diagnosis, saying "He wasn't expected to live for more than 4-5 years and we were told there was nothing we could do apart from loving him to the fullest" (*About* Yash Gandhi Foundation, n.d.). Everybody loved Yash, but there are no words to describe the love that he spread. His perseverance and joy touched so many people. Despite his illness, Yash was eager to try new activities, some of his favorite being music classes, face painting, and playing with a stuffed Elmo from *Sesame Street.* The invaluable lessons Yash taught his friends and family every day inspired Ashesh and Sonal Gandhi to search for a cure. In 2001, they created the Yash Gandhi Foundation, a 501(c)(3) non-profit organization committed to "rais[ing] awareness, build[ing] patient advocacy, and sustain[ing] research efforts into finding a cure for Mucolipidosis II (I-cell disease)" (*About* Yash Gandhi Foundation, n.d.). Yash passed away several years ago. While I only have a few distinct memories of Yash (**Figure 1**), I am inspired by the stories I hear about his amazing character and hope to support the Yash Gandhi Foundation by raising awareness for MLII.

Figure 1: Image circa 2006. From left to right: Simi Gandhi, Yash Gandhi, Kavi Gandhi (Yash's brother), and Sia Gandhi (Simi's twin sister).

Epidemiology and Prognosis

In the US, a disease is considered to be rare if it impacts less than 200,000 Americans (*Rare Disease Day*, 2019). Approximately 7000 to 10,000 known rare diseases affect 25 to 30 million

Americans (*NIH study*, 2021). MLII is a rare autosomal recessive disease affecting approximately two in every million children (*About* Yash Gandhi Foundation, n.d.). Autosomal recessive inheritance occurs when both parents carry a mutated gene and both copies are expressed in the affected offspring. MLII is characterized as a lysosomal storage disorder that affects the body's ability to break down certain fats. Multiple enzyme deficiencies lead to the buildup of fats and complex carbohydrates within cells and tissues. This accumulation leads to progressive damage to cells and organs, causing most patients with MLII to pass away before their tenth birthday. (*About* Yash Gandhi Foundation, n.d.). Currently, there is no cure for MLII.

Biochemical Pathway in Normal Cells

Lysosomes are organelles that break down and dispose of intracellular waste. In healthy cells, lysosomes use proteins called lysosomal enzymes to catalyze the degradation of waste materials. Lysosomal enzymes are synthesized in the endoplasmic reticulum (Gandhi K., 2021). Once synthesized, lysosomal enzymes travel to the Golgi apparatus, where they are labeled with residues of signaling molecule mannose-6-phosphate (M6P) (van Meel *et al.*, 2016). M6P is responsible for signaling the transportation of lysosomal enzymes to the lysosome (Kang *et al.*, 2018*)*.

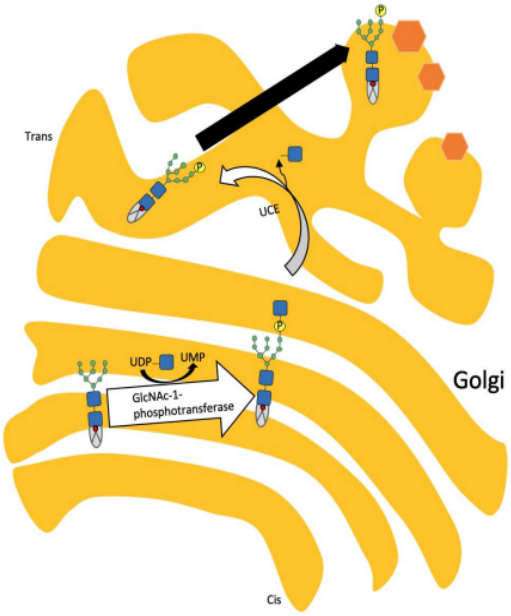


Figure 2: The diagram shows the addition of a phosphate group to mannose in the cis-Golgi network with the help of GlcNAc-1-phosphotransferase. Additionally, the figure depicts the removal of GlcNac to expose M6P residues in the trans-Golgi network (Khan & Tomatsu, 2020).

UDP-N-acetylglucoseamine-1-phosphotransferase enzyme (GlcNAc-1-phosphotransferase) is responsible for producing signaling molecule M6P. (*I Cell Disease*, n.d.). This process occurs when GlcNAc-1-phosphotransferase adds a phosphate group to certain mannose residues located on lysosomal enzymes in the cis-Golgi network. Then, uncovering enzyme, N-acetylglucosamine-1-phosphodiester α-N-acetylglucosaminidase, removes the N-acetylglucosamine molecule (GlcNAc), leaving the M6P residues exposed in the trans-Golgi network (**Figure 2**). Lysosomal enzymes labeled with M6P normally

act as a high affinity ligand to M6P receptors (MPRs) located in the trans-Golgi network. When M6P binds to a MPR, M6P is able to carry out its function, directing lysosomal enzymes to the lysosome and the cell properly disposes of its waste (van Meel *et al.*, 2016).

Genetics

GlcNac-1-phosphotransferase is a $\alpha_2\beta_2\gamma_2$ heterohexamer and is normally encoded by two genes. The *GNPTAB* gene encodes the α subunit and the β subunit which are typically responsible for recognizing lysosomal enzymes. MLII concerns mutations in the *GNPTAB* gene that encodes for the α and β subunits of GlcNac-1-phosphotransferase (Qian *et al.*, 2015).

In patients with MLII, a mutation in *GNPTAB* gene located on the long arm of chromosome 4 (4q21-q23) leads to a deficiency of GlcNAc-1-phosphotransferase (*I Cell Disease*, n.d.). Velho *et al.* reported 258 known mutations in the *GNTAB* gene that lead to deficient levels of the enzyme GlcNAc-1-phosphotransferase. Such deficiency is caused by varying types of mutations, including frameshift mutations (39%), missense mutations (26%), nonsense mutations (23%), splice defects (9%), and deletions, insertions, and duplications (3%) (Velho *et al.*, 2019).

The most common type of mutation that causes MLII is a homozygous frameshift mutation. A frameshift mutation occurs when the number of nucleotides in a DNA sequence is changed. This can occur when one or more nucleotides is added or deleted from the sequence, consequently producing an abnormal sequence of nucleotides. A recent review of 516 MLII cases revealed that the most common pathogenic variant in patients causing complete loss of function of GlcNAc-1-phosphotransferase was c.3503_3504 del (**Figure 3**).

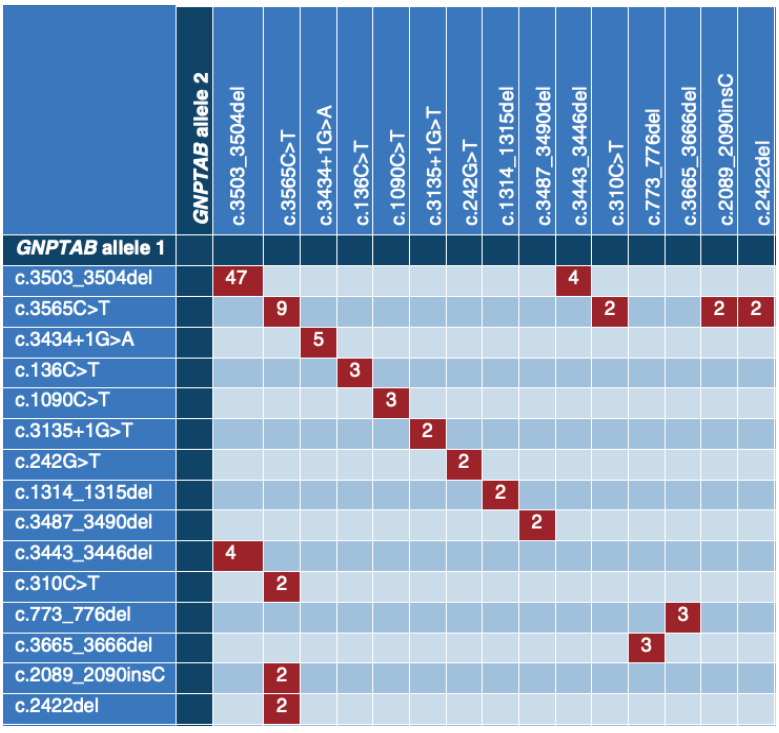


Figure 3: Pathogenic variants in children diagnosed with MLII. The number in each box represents the number of patients with a specific combination of mutations. For example, 47 children with MLII had a c.3503_3504 del mutation (Adapted from Dogterom *et al.*, 2021).

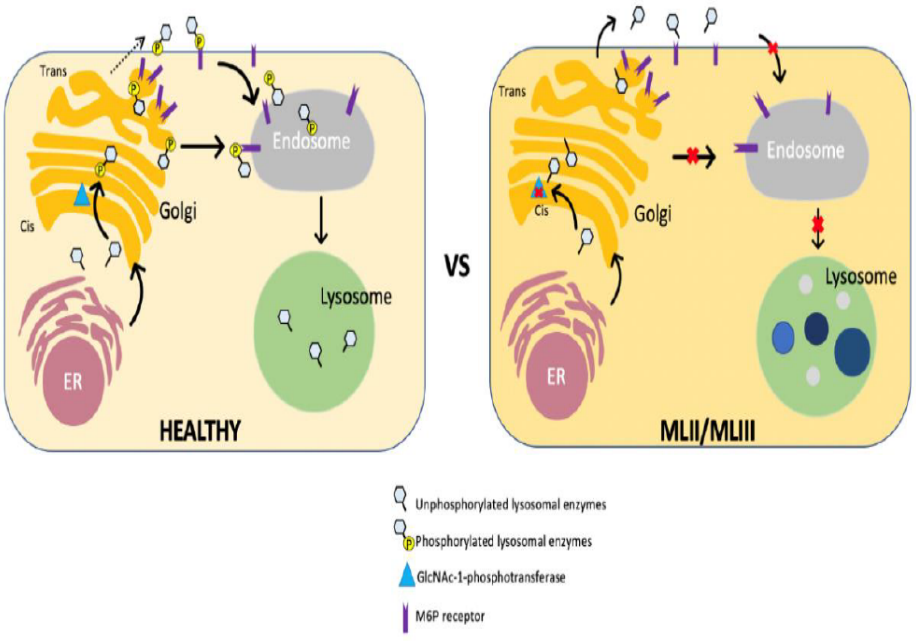
The c.3503_3504 del notation refers to the specific location of the deletion on the *GNTAB* gene, with "c" indicating the coding region of the gene, 3503 and 3504. This deletion mutation

leads to a shift in the sequence of amino acids in the protein produced by the gene. The specific consequence of this shift is frameshift mutation Leu1168Glnfs*5, involving the substitution of the amino acid leucine with glutamine. The “fs*5” indicates that it is a frameshift mutation occuring five nucleotides before the original stop codon resulting in a premature stop codon. This means that the essential protein, GlcNAc-1-phosphotransferase will not be synthesized in patients with MLII (Dogterom *et al.*, 2021).

Genetic Impact on Biochemistry in MLII

As noted previously, mutations in the *GNTAB* gene cause a deficiency in the enzyme GlcNAc-1-phosphotransferase. Lack of this enzyme results in the inability to add a phosphate group to mannose residues located on lysosomal enzymes in the cis-Golgi network (Khan & Tomatsu, 2020). Thus, signaling molecule M6P is no longer produced in children with MLII (*I Cell Disease*, n.d.). Without M6P, lysosomal enzymes no longer have directions to get to the lysosome. This leads to a buildup of cholesterol, phospholipids, glycosaminoglycans, and other waste in the lysosome as well as an influx of lysosomal enzymes in the bloodstream, hence the onset of lysosomal storage disorder (Gandhi K., 2021; Khan & Tomatsu, 2020). Ultimately, lysosomes in children with MLII are dysfunctional, causing fatty acids (mucolipids) and complex carbohydrates (mucopolysaccharides) to accumulate in the cells of many tissues (**Figure 4**). This results in the clinical manifestations and failure to thrive in patients with MLII. (*I Cell Disease*, n.d.).

Figure 4: Healthy cells show optimal activity of enzyme GlcNAc-1-phosphotransferase, allowing for the phosphorylation of lysosomal enzymes. Consequently, lysosomal enzymes are effectively trafficked to the lysosome with the help of M6P, ridding the cell of toxic metabolites. In MLII cells, enzyme GlcNAc-1-phosphotransferase is deficient, leaving mannose residues unphosphorylated. This creates an absence of M6P, meaning lysosomal enzymes are not directed to the lysosome. Toxic metabolites accumulate intracellularly while lysosomal enzymes build up outside of the cell (Khan & Tomatsu, 2020).



Diagnosis

Based on failure to thrive, developmental delays,

clinical features, and genetic evaluation, Yash was diagnosed with MLII at 11 months of age. Most patients exhibit symptoms at birth whereas others begin to show symptoms at 6-10 months of age. If a child is believed to have MLII, specialists often assess the measurement of GlcNAc-1-phosphotransferase in white blood cells (*I Cell Disease*, n.d.). However, this mechanism is not available in many countries (Khan & Tomatsu, 2020). Diagnosis can be confirmed as MLII if the child has elevated serum levels of certain enzymes including B-hexosaminidase, iduronate sulphatase, and arylsulphatase A. A specific pattern of lysosomal enzyme deficiency in the fibroblasts also confirms the diagnosis of MLII. (English & Ettedgui, 2009). A prominent phenotypic manifestation of MLII is the swelling of the gums (gingival hyperplasia) due to a lack of the enzyme cathepsin L. MLII can be difficult to diagnose because the symptoms are similar to that of other lysosomal storage disorders (Khan & Tomatsu, 2020).

Clinical Manifestations

The buildup of waste in the cells of patients with MLII manifests in many phenotypic changes. Waste molecules accumulate in the brain, which leads to delayed neurological development. Influx of waste in the visceral organs enlarges the spleen and liver and impairs their function. The heart is also larger than normal. Furthermore, structural changes in the skeleton are common in children with MLII. Babies with MLII are often born with abnormally shaped bones (bone dysplasia) and fractured bones. After the first one to two years of life, children are unable to grow. Other serious skeletal manifestations include premature fusion of bones in the skull (craniosynostosis), loss of bone mineral density (osteopenia), neonatal hyperparathyroidism leading to increased levels of calcium in the blood, abnormalities of the thoracic spine, hip dysplasia, and the hardening and shortening of tendons and muscle in all major joints. As an MLII patient gets older, there is a curvature of the spine known as kyphoscoliosis. The combination of these skeletal changes limits mobility in children with MLII (Khan & Tomatsu, 2020).

Changes in the facial structure, along with the progressive accumulation of metabolic substrate in the larynx, epiglottis, and trachea, causes the narrowing of the airway. This results in breathing complications while the patient is asleep (obstructive sleep apnea). Patients are prone to recurrent respiratory tract infections. The progressive narrowing of the airway is generally the leading cause of death for children with MLII. (Khan & Tomatsu, 2020).

Management of MLII

Presently, there is no cure for MLII. Patient care is currently confined to management of symptoms and prevention of respiratory illness. Surgical procedures such as hip replacement can decrease bone pain in patients. Physiotherapy can also drastically improve the quality of life of children with MLII. Clinical trials of bisphosphonate therapy in children with MLII show promising alleviation of osteopenia.

Bisphosphonate therapy works by inhibiting the osteoclast, thus preventing the breakdown of bone material. Antibiotics can be used to prevent bacteria from entering the bloodstream and traveling to the heart (bacterial endocarditis) (Khan & Tomatsu, 2020).

Hematopoietic Stem Cell Transplantation (HSCT) has shown mixed results. This procedure aims to provide MLII patients with donated cells that have the typical ability to chaperone lysosomal enzymes to the lysosome in order to perform the essential function of degrading intracellular waste. Patients who received HSCT treatment did not tend to live any longer than patients without treatment (Lund *et al.* 2014). Enzyme Replacement Therapy and gene therapy may be beneficial in the management and/or cure of disease, but are not yet fully studied. The addition of pharmacological chaperone proteins to the body as well as exon skipping mechanisms (**Figure 5**) are both potential cures, but are insufficiently researched at the moment (Khan & Tomatsu, 2020).

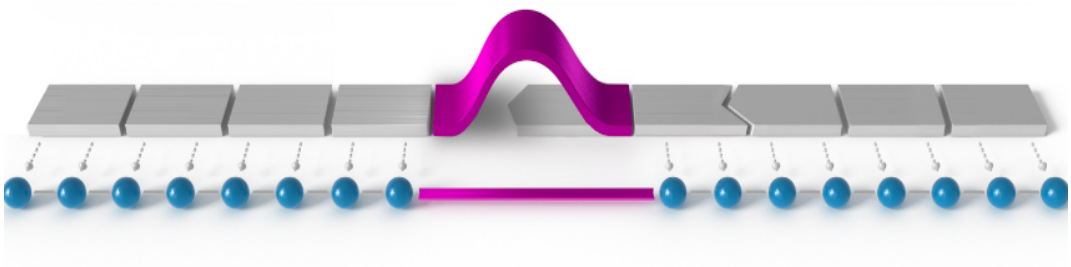


Figure 5: Exon skipping is a mechanism by which specific exons, or parts of the gene, are skipped resulting in the production of a modified protein. This technique is a potential strategy for treating MLII. "Skipping" over the mutations could possibly produce the partially or fully functional enzyme, GlcNAc-1-phosphotransferase (Matos *et al.*, 2020). Image courtesy of Sarepta Therapeutics.

Pre-clinical research efforts regarding MLII have been furthered tremendously in the past few decades. One example of this is the University of Pennsylvania's ML cat colony. MLII occurs naturally in cats. Affected cats exhibit many of the same phenotypic traits as humans with MLII. This makes them an important model in better understanding the disease and potentially finding a cure. Researchers at the University of Pennsylvania are currently expanding the MLII cat colony in attempts to further I-cell research using the feline cat model (Gandhi K., 2021).

Family Support for MLII

While research is in progress, patients and their families need tremendous support. The Yash Gandhi Foundation has provided that support for MLII families all over the world. In the Winter of 2012, my father and his side of the family traveled to Tanzania to climb Mt. Kilimanjaro to raise awareness and funds for MLII research. Most of them grew up in the neighboring country of Kenya. Shortly before the climb, Ashesh Gandhi wrote "You can imagine the emotional journey of returning to your birthplace,

seeing where you grew up, remembering some of your fondest childhood experiences, all the while reminded that the simple things you enjoyed as a child were things Yash could never do. It will fuel the fire of passion in our climbers during their 50-kilometer hike up the Machame Route to the Uhuru Peak" (Gandhi A., 2012). Upon reaching Uhuru Peak, the adventurers placed Yash's Elmo plushie in a wooden box where climbers often leave memories of their trip (**Figures 6 & 7**).

Figure 6 (top): Gandhi crew reaches Uhuru Peak at 19,354 feet.
Figure 7 (bottom): Yash's favorite Elmo toy is placed in Mt. Kilimanjaro's memory box.

Today, the Yash Gandhi Foundation often hosts 5K fundraisers. The organization encourages viewers of their website to raise awareness for rare diseases. My cousin, Kavi, also runs the organization's social media pages, including a private Facebook group, offering moral support for parents and siblings of patients with MLII. The Summer before his senior year in high school, Kavi completed an internship at Greenwood Genetic Center in South Carolina, a premier lab for ongoing MLII research.

Although the prognosis of MLII is poor, Yash and many other patients teach loved ones every day to not take laughter, smiles, and quality time together for granted. It is in this spirit that our family continues to search for a cure for MLII so that other children may have the chance to share more joyful memories with those they love. Yash's story is just one key example of why research for rare disease is so important. Regardless of how many people MLII affects, every child deserves to experience life at the highest quality. As supporters of the Yash Gandhi Foundation and the rare disease community say, "Alone we are rare. But together, we are strong" (*About* Yash Gandhi Foundation, n.d.).

References

Dogterom, E. J., Wagenmakers, M. A., Wilke, M., Demirdas, S., Muschol, N. M., Pohl, S., Meijden, J. C. van der, Rizopoulos, D., Ploeg, A. T. van der, & Oussoren, E. (2021). Mucolipidosis type II and type III: a systematic review of 843 published cases. *Genetics in Medicine*, *23*(11), 2047-2056. https://doi.org/10.1038/s41436-021-01244-4

English, R. F., & Ettedgui, J. A. (Eds.). (2009). *Paediatric Cardiology* (3rd ed.). ScienceDirect. https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/i-cell-disease

Gandhi, A. (2012, December 9). Climb to Kilimanjaro - journey of a lifetime. *Welcome to Yash Gandhi Foundation*. http://ygf4icell.blogspot.com/2012/12/climb-to-kilimanjaro-journey-of-lifetime.html

Gandhi, K. (2021, May). *Mucolipidoses II diagnoses handbook*. https://www.ygf4icell.org/_files/ugd/af3d2a_c2e52df5e78f4ac39b91f04995434d6f.pdf

Kang, J.-Y., Shin, K. K., Kim, H. H., Min, J.-K., Ji, E. S., Kim, J. Y., Kwon, O., & Oh, D.-B. (2018). Lysosomal Targeting Enhancement by Conjugation of Glycopeptides Containing Mannose-6-phosphate Glycans Derived from Glyco-engineered Yeast. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-26913-4

Khan, S. A., & Tomatsu, S. C. (2020). Mucolipidoses overview: Past, present, and future. *International Journal of Molecular Sciences*, *21*(18), 6812. https://doi.org/10.3390/ijms21186812

Lund, T. C., Cathey, S. S., Miller, W. P., Eapen, M., Andreansky, M., Dvorak, C. C., Davis, J. H., Dalal, J. D., Devine, S. M., Eames, G. M., Ferguson, W. S., Giller, R. H., He, W., Kurtzberg, J., Krance, R., Katsanis, E., Lewis, V. A., Sahdev, I., & Orchard, P. J. (2014). Outcomes after hematopoietic stem cell transplantation for children with i-cell disease. *Biology of Blood and Marrow Transplantation*, *20*(11), 1847-1851. https://doi.org/10.1016/j.bbmt.2014.06.019

Matos, L., Vilela, R., Rocha, M., Santos, J. I., Coutinho, M. F., Gaspar, P., Prata, M. J., & Alves, S. (2020). Development of an antisense oligonucleotide-mediated exon skipping therapeutic strategy for mucolipidosis ii: Validation at RNA level. *Human Gene Therapy*, *31*(13-14), 775-783. https://doi.org/10.1089/hum.2020.034

National Institutes of Health. (2021, October 22). *NIH study suggests people with rare Diseases face significantly higher health care costs* [National Institutes of Health]. N. Retrieved January 31, 2023, from https://ncats.nih.gov/news/releases/2021/nih-study-suggests-people-with-rare-diseases-face-significantly-higher-health-care-costs

National Organization for Rare Disorders. (2019). *Rare Disease Day: Frequently asked questions* [Infographic]. https://rarediseases.org/wp-content/uploads/2019/01/RDD-FAQ-2019.pdf

NORD Member Organizations. (n.d.). *I Cell Disease*. *Rare Disease Database*. Retrieved January 8, 2023, from https://rarediseases.org/rare-diseases/i-cell-disease/#:~:text=I%2Dcell%20disease%20(mucolipidosis%20II,than%20those%20of%20Hurler%20syndrome.

Qian, Y., van Meel, E., Flanagan-steet, H., Yox, A., Steet, R., & Kornfeld, S. (2015). Analysis of mucolipidosis ii/iii GNPTAB missense mutations identifies domains of udp-glcnac:lysosomal enzyme glcnac-1-phosphotransferase involved in catalytic function and lysosomal enzyme recognition. *Journal of Biological Chemistry*, *290*(5), 3045-3056. https://doi.org/10.1074/jbc.M114.612507

Sarepta Therapeutics. (n.d.). *Leveraging RNA technology to treat rare disease*. (n.d.). Sarepta Therapeutics. Retrieved January 31, 2023, from https://www.sarepta.com/science/rna-platform

van Meel, E., Lee, W.-S., Liu, L., Qian, Y., Doray, B., & Kornfeld, S. (2016). Multiple domains of glcnac-1-phosphotransferase mediate recognition of lysosomal enzymes. *Journal of Biological Chemistry*, *291*(15), 8295-8307. https://doi.org/10.1074/jbc.M116.714568

Velho, R. V., Harms, F. L., Danyukova, T., Ludwig, N. F., Friez, M. J., Cathey, S. S., Filocamo, M., Tappino, B., Güneş, N., Tüysüz, B., Tylee, K. L., Brammeier, K. L., Heptinstall, L., Oussoren, E., Ploeg, A. T., Petersen, C., Alves, S., Saavedra, G. D., Schwartz, I. V., . . . Pohl, S. (2019). The lysosomal storage disorders mucolipidosis type ii, type III alpha/beta, and type III gamma: Update on GNPTAB and GNPTG mutations. *Human Mutation*, *40*, 842-864. https://doi.org/10.1002/humu.23748

Yash Gandhi Foundation. (n.d.). *About*. Yash Gandhi Foundation. Retrieved January 31, 2023, from https://www.ygf4icell.org/

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