Solving Lactase Non-Persistence with LacZ AAVs

A paper by Soham Kuvalekar, Aswath Balaji & Aayush Mehrotra.
Class 3A, Gymnasium Kirschgarten

Lactase non-persistence, aka. lactose intolerance, is a widespread problem due to the increase of lactose sugars in our diet, affecting up to 65-70% [3] of the world's adult population. Lactase non-persistence occurs after the waning period around the age of five [2], marking the decline in production of glycoside hydrolase family 35 (GHF-35) β-galactosidase [1, 15], the lactose digesting enzyme in humans. This reduction in enzyme production is what causes the beginning of lactose intolerance, making it more challenging to enjoy simple dairy products such as a cheesy pizza, without causing discomfort.

A promising treatment method is gene therapy, which can semi-permanently enable lactase production by inserting DNA into the intestinal epithelial cells. An optimal solution for the production and delivery of the DNA is still debated upon. The mass-production of such a treatment would allow a significant percentage of the population to consume modern lactose-containing food products without the need for supplements, reducing anxiety and increasing quality-of-life.

1. Preface

What is our motivation to work on the topic we chose?

Lactose non-persistence is a widespread problem due to the increase of lactose in our diet. Depending on your genes, you may be affected by lactose intolerance at a different severity. The possibility of dispelling this problem by simply taking a pill is extremely exciting for us!

What is especially interesting?

We are especially fascinated by the process of entry in our therapy, where DNA that **humans** engineer manages to assimilate itself into the genome of someone who consumes a pill. Previously, we envisioned gene therapy as a complex procedure involving advanced technologies beyond imagination, rather than a method as straightforward as this.

What are our questions with respect to the chosen topic?

To address the issues regarding lactase non-persistence, we will first need to understand the functioning of lactase non-persistence, more precisely, what lactase persistence does in contrast to lactase non-persistence. Then to treat lactase non-persistence, we will answer the question of how to reintroduce lactase using gene therapy. Finally, looking at the problem in the big picture we will discuss if and how the mass production of the solution found could work and if such a solution is effective.

2. Introduction

In this method, we will insert engineered DNA into the cells that line the small intestine, allowing the body to produce (EC-)β-galactosidase enzyme encoded from the LacZ gene to help in digesting lactose.

Gene therapy is mostly used in the fields of genetic medicine, with the aim to improve overall health of the people by correcting genetic disorders. In our topic of lactose intolerance, gene therapy could enable people to consume foods containing lactose, without having to take supplements or experiencing discomfort.

Even though gene therapy looks quite promising for the future, alternative treatments for lactose intolerance also exist. These include dietary changes, such as consuming less lactose containing foods or taking lactase enzyme supplements before consuming any dairy products. Along with that, probiotics and prebiotics are being explored for their potential to ease lactose intolerance symptoms by making our gut healthier. However, when compared to gene therapy, these alternative treatments only offer temporary relief and do not fix the cause of lactose intolerance as well as gene therapy does.

3. Description of engineering technique

The goal of the gene therapy that we chose to cover as a group, is to cure lactose intolerance. In scientific terms, the goal of the gene therapy would be to reintroduce lactase enzymes into the intestinal systems of human beings. This would then allow a person who previously could not digest lactose, to be able to do so. The process was originally done by a university student who described it in great detail [14], which we will now explain here again.

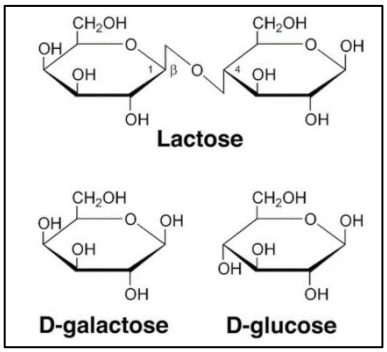

Figure 1: Diagram showing structures of Lactose and its base components. All the enzymes do is break the bond in between them.

Few studies and experiments [4] have been run in the past to use gene therapy to correct lactase non-persistence. One of these few is using an oral viral vector with coding sequences of Lactase enzymes to induce a long-term solution to lactase deficiency. We will describe the scientific parts of these in more detail further down this paper.

Lactose is a complex sugar, made up of two subunits (a disaccharide). The role of Lactase is to break these two units up. These subunits can then be easily digested.

Although humans start out being able to produce Lactase in their small intestines, which allows them to digest Lactose, using an gene called LacZ from E. Coli bacteria leads to the same digestive capabilities.

LacZ encodes EC-β-galactosidase (GHF-2) [16], an intracellular enzyme that cleaves the disaccharide lactose into glucose and galactose. LacZ is part of a Lac Operon, which is something we learnt about in biology class. It is how the bacteria adapts to be able to digest lactose in case it encounters any.

The goal of the gene therapy is to use an Adeno Associated Virus (which is a “defective” virus, and non-pathogenic in humans and other species), to deliver the LacZ enzyme into the intestinal tract of an organism. The effectiveness of this method has already been empirically proven in rats [4].

Adeno Associated Viruses can be found in almost all tissue samples that have ever been tested and are the gold standard in safety and reliability in gene therapy. They cannot replicate on their own, and the DNA that we place inside it combines with the host’s genome in safe and predictable ways.

We engineer these Viruses and decide what DNA ends up inside them with an ingenious system. We work with DNA molecules called plasmids, which we can engineer to suit our needs. It is these plasmids that contain the DNA needed to not only build the shell of the virus, but also whatever we choose to put inside it as well.

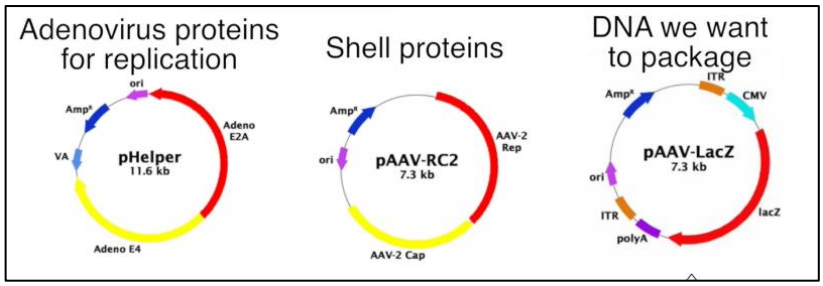

Figure 2: Diagram of structure of the plasmids used in the therapy.

When these plasmids naturally find their way to the nucleus of a cell, they get translated into RNA, and the corresponding proteins and amino acids are built. This means if we set up the plasmids of an AAV correctly, we will be able to get it to not only translate and then transcribe the DNA required to make the LacZ enzymes, but also get it to self-replicate, thereby extending the efficacy of the therapy by a significant time.

The pHelper and PAAV-RC2 helper plasmids are less interesting for pure gene therapy. These are the plasmids that allow the Virus to survive and replicate. Both have a purple "ori" sequence, which is analogous to a primer (Origin of Replication). They also have a deep blue Amp gene, which codes for Ampicillin resistance. Both of these allow us to put the gene into a bacterial cell and allow it to replicate and provide us with sufficient copies for the therapy.

Let us turn our focus to the third plasmid. We are already familiar with the roles of the ORI and the Amp sequences. The two orange "ITR" sequences serve as further indicators, that whatever material is between these Inverted Terminal Repeats must be packaged into the shell of the virus, and thereby activated. The light blue "CMV" sequence is a promoter, which encourages proteins to bind to it, and transcribe the following red lacZ DNA into RNA. The purple "polyA" signal is also simply an essential part of gene therapy with Adeno Associated Viruses. For our example, the CMV promoter was used, with a sv40 polyA signal.

With these three essential plasmids, all we have to do is to allow these plasmids to enter a group of mammalian cells, wait for the plasmids to create numerous copies of the virus, extract the virus from these mammalian cells, and then prepare them for oral consumption.

Figure 3: Picture of capsules containing the finished therapy.

The logistical side of the preparing these plasmids into a finished therapy is long, very detailed, exhaustive, and exhausting. We will not be going over all the details in the interest of time.

What is important is that the process is carried out in sterile conditions. The plasmids are added to mammalian cells which float in a nourishing media and allowed to culture. The mixture is then combined with a miniscule substrate, sedimented, centrifuged, and purified by various other means in order to extract the virus, and the extracted virus is then combined with cellulose and packaged in gel capsules, ready for consumption.

4. Documentation and pictures of research institutions visited

Figure 4: Picture of Prof.Dr. van Nimwegen.

In order to gain valuable insights from a professional on our topic, we contacted many professors from our very own University of Basel. We had a few responses, but the only professor who managed to give us an interview was Prof.Dr. Erik van Nimwegen.

With an astoundingly high h-index of fifty-eight, we found our interview with Prof.Dr van Nimwegen both informative and entertaining. His fascinating research into the functioning and evolution of genome regulatory functions provided him with the sufficient expertise to answer all of our questions in a very satisfying manner.

Our interviewee started his scientific career off as a theoretical physicist, which means that he understandably does not have that much experience in an actual wet lab. This works out well for us, as, as you will read in the Question and Answers ahead, our project is very much still in its theoretical stage as of now. This is also why we only toured the spectacular office spaces of the Biozentrum Basel, and only a smaller one of its various labs.

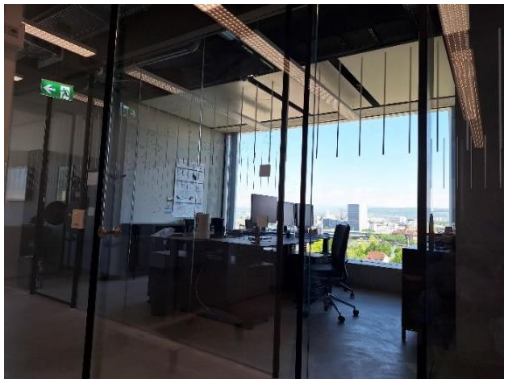

Figure 5: An Office in the higher floors of the Biozentrum

Figure 6: Picture of one of the labs in the Biozentrum

Questions and Answers

The interview itself lasted around 90 Minutes. We gleaned so much knowledge from it that it would be impossible to include it all in this paper. Hence, we will present the questions and their answers in truncated forms, which contain the essence of what matters and no loss of information.

Q1. It is surprising to see a virus, which is normally a bad thing, be used in such a useful way. Are such cases common in medicine?

Viruses are nothing more than spontaneously occurring parasites. One cannot call a knife “bad” because a murderer uses one, nor can it be called “good” when in hands of a chef. Viruses have evolved certain tricks over the years to get into the genome of a host and create certain proteins to survive, which is something that independently evolved in nature. It is “natural,” and not good or bad. It is a very complicated thing to use these evolved tricks for our benefit in medicine in the form of such gene therapies, and that’s why research is even currently being done on it.

Q2. Are there any other ways for such gene therapies to be administered apart from viruses?

Non-viral means are also possible, yes. Research is being done into small organic vesicles which serve the same purpose. But it is important to understand that such gene therapies can be done with essentially anything. At its essence, Gene Therapy is simply placing DNA into a host genome by any means necessary. Bacteria, for example, do such "gene therapies" all the time. Their main means of evolution is simply taking in any DNA they encounter and making it part of their genome! It is just that, due to what we discussed in Q1, Viruses are particularly good at gene therapies due to their evolved mechanism, and it makes the most sense to use them for now.

Q3. After engineering the plasmids with the LacZ DNA, how does the host cell know where to send these newly produced Lactase enzymes?

AAVs are special, because it is easy to control where they end up in the genomic sequence of the host. As far as I know, promoters are used in plasmid engineering to ensure that the respective processes happen in the right place. The enzyme might not even "need" to go to a specific place. After the DNA is built into the genome, the LacZ is simply produced, and breaks down any Lactose it encounters.

Q4. Are there any other ways to produce a lasting gene therapy for lactase persistence in humans?

One of the key issues faced by people with Lactase non-persistence takes place in the large intestine. Without the necessary enzymes to break Lactose down, bacteria in the large intestine which normally do not get access to energy rich food now have a feast in the form of lactose sugars. This is what causes bloating, indigestion, and other issues. Henceforth, a solution to the problem may be to simply address the problem at its source. One could modify these bacteria to be unable to digest Lactose, or even simply remove them from the human microbiome. However, such modifications usually carry huge ramifications, and it is extremely hard to predict the result safely. One must be careful in such situations!

Q5. Would such a gene therapy with cellulose digesting enzymes theoretically allow humans to eat wood, like termites?

Well, primarily, these two situations are not the same. All it takes is one enzyme to break Lactose into smaller parts that are already normally digestible. Cellulose, on the other hand, might need to be broken down by multiple enzymes before it is easily digestible by our bodies. It would, in theory, work. However, it is far more likely that humans genetically engineer tree bark to be composed of materials our bodies can naturally digest at this stage, before such an outlandish scenario happens!

Q6. Why are these sorts of gene therapies not as widespread as cough syrup if they are so useful?

There is one simple answer to this question, and it is safety. Over-the-counter medications such as cough syrup have been professionally researched, and the ramifications and side effects they cause are well-documented and controlled. The inherent risk that is involved in gene therapy is simply too high! Maybe, as research gets better in the future regarding them, they may be more widespread, but certainly not at this point in time.

Q7. What are your views regarding the ethics of gene therapy as a whole?

It is especially important to distinguish between "Doing something" and "Knowing how to do something." Taking action on something is often dangerous to even think about, and that is why I can safely say I do not have a personal opinion on this part of ethics. It is a gray area for a reason. **Knowing how to do something**, on the other hand, is very important, and I am all for it. Researching is inherently a positive thing. It allows us to identify problems that may have easy and reliable fixes and improve human quality of life without negatively affecting anything. Research in gene therapy is going to be done regardless of the ethics behind it. This is why it is important that we set ground rules and support research for good.

Q8. What difficulties are there trying to understand the functioning of a DNA sequence? Is it possible to predict and deal with side-effects?

The study of DNA and the Genome is, even today, an insanely cryptic and complicated branch. The problems that one tries to deal with are often very nuanced, and the solutions we tend to come up with are brash in comparison. Genes are often expressed in diverse ways due to tiny variables known as transcription factors which promote and demote gene expression (which is coincidentally my field of study). **Blowing up the Mittlere Brücke** to reduce foot traffic in Basel **may** theoretically achieve its intended goal but will undoubtedly cause catastrophes that are impossible to even plan for. It is exceedingly difficult to predict and deal with such side-effects.

Q9. Is the mass production of such gene therapies feasible?

In fact, the MRNA covid vaccine works quite similarly to gene therapies. A gene sequence is produced as a bit of a virus, which helps us build up immunity to the actual thing. That was mass produced quite efficiently, so I do not see why other gene therapies the exception should be!

Q10. Would it be ill-advised for a student like us to try and recreate these sorts of therapies in their school's lab, perhaps for their Maturaarbeit?

Absolutely, by no means at all, never, ever try such a gene therapy on yourself! I have already explained to you how difficult this entire field of study is. I do not think I could even justify trying such therapies out on lab-rats unless you absolutely know what you are doing. Always stay on the safer side of such things!

5. Discussion

Using AAV vectors to introduce the LacZ gene, this technique has successfully demonstrated the feasibility of treating lactase non-persistence through direct DNA insertion into intestinal epithelial cells in humans [14]. Although the test size is extremely small, no side effects have been observed to date.

Further research steps would involve finding alternative vectors as a transport mechanism for LacZ. The preparation of AAV requires penicillin-streptomycin, to which some people are allergic, and residual proteins may be left behind, which could lead to side effects if not properly removed. To address these issues, an alternative vector, Chitosan, appears to be more suitable for this application [5]. Chitosan, like AAV, is orally consumable and much easier to work with. Its cost per gram is extremely low, and it requires no intermediate mammalian cells for reproduction. Using Chitosan would make gene therapy for lactase non-persistence more viable for mass production.

Using gene therapy as a treatment for lactase non-persistence offers multiple benefits. It has a long-lasting effect, is easily ingested orally, and is highly effective, almost entirely eliminating the need for external lactase pills. However, there is a severe lack of clinical testing, and it is of utmost concern to conduct such testing before releasing such treatments to the larger public. Allergic effects, drastic negative changes in the gut microbiome, and severe side effects due to, but not limited to the difference in β-galactosidase family are among the numerous concerns. The use of viruses to administer LacZ is also a consideration, but is of lesser concern, since AAVs have an excellent track record [6,7].

Apart from the papers referenced in this study, there has been little research on the treatment of lactase non-persistence using gene therapy. Such a treatment would allow 65-70% [3] of the world's adult population to permanently reintroduce lactose into their diets. Currently, whey containing lactose is a waste byproduct of the milk industry [8] and is often used as a filler in many food products. This creates a seemingly invisible barrier to consumption for a substantial percentage of the population. Asian populations would particularly benefit from such treatments, as their historically primary sources of nutrition did not include milk, resulting in only 12.1-32% LP [10].

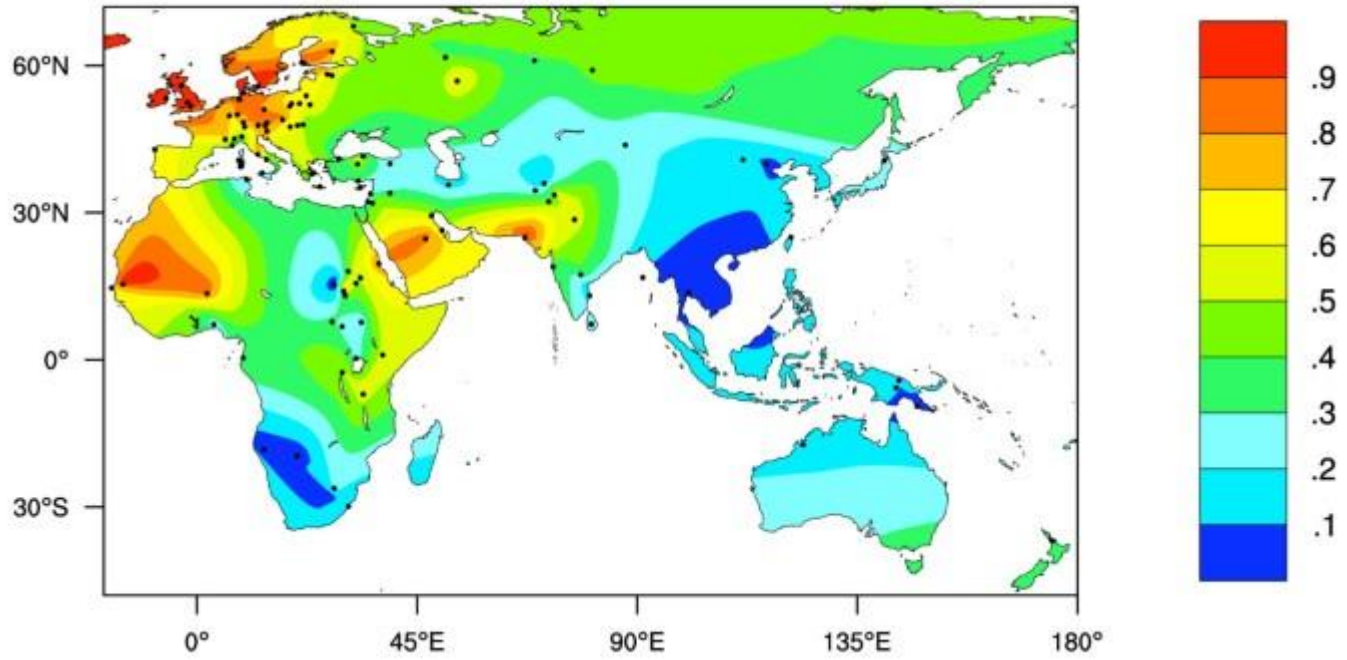

***Figure 7: Interpolated map of Old-World LP (Lactase Persistence) phenotype frequencies**. Dots represent collection locations. Colours and colour key show the frequencies of the LP phenotype estimated by surface interpolation. [Source: 9]*

Looking at gene therapy in the big picture, it is an extremely powerful tool. At the same time taking gene therapy to its extremes is questionable. Underestimating genetic modification could lead to catastrophic biohazards. It could also be used in ways which could be morally questionable, such as modifying human fetuses to match their parents' wishes. To take such risks is inexcusable, as the lives of many people are at stake. On the other hand, the potential benefits may even be lifesaving.

The Covid-19 vaccine can be classified a gene therapy product [11]. Using mRNA, it makes cells of the vaccinee produce antigens for SARS-CoV-2. The adverse effects of the vaccination were mild to moderate [12] but did not affect daily activities. Extrapolating from the Covid-19 vaccine, which proved to be invaluable, to using gene therapy for other treatments on a large scale, could prove to be equally effective.

Successful gene therapies until now are the Covid-19 vaccine, Severe combined immune deficiency (SCID), several metabolic disorders and sickle cell disease to list a few. [13] Their respective clinical trials have shown very promising results. This shows that, in general, gene therapy can give treatments in situations where any other treatment is ineffective or only effective to a certain small extent.

Considering all the concerns and benefits of this treatment and its derivatives, we anticipate an overall net-positive effect with little to no negative consequences. The treatment of the intestinal epithelial cells using gene therapy is local, personal and does not spread, thus it will not affect more than one person. Even so, clinical trials are unavoidable and of absolute necessity. Risking any life is not worth any risk at all.

6. Summary

In this paper we investigated the feasibility and potential of gene therapy to treat lactose intolerance/lactase non-persistence, a condition that affects a large amount of the population around the world. By using gene therapy, we can place specific DNA (LacZ) into our intestinal cells, which will then provide an alternative to the human GHF-35 β-galactosidase enzyme. This process involves the use of harmless viruses called Adeno Associated Virus vectors, to deliver the DNA into the cells, which then produce the required enzymes. Although this therapy does allow individuals to reintroduce lactose-containing foods into their diets, we still know relatively little about future side effects. Hence, further research is still needed, and ethically encouraged to fully understand the effectiveness and safety of this therapy, before it can be used on those, affected by the condition.

7. Sources

1. Anguita-Ruiz, A., Aguilera, C. M., & Gil, Á. (2020). Genetics of Lactose Intolerance: An Updated Review and Online Interactive World Maps of Phenotype and Genotype Frequencies. Nutrients, 12(9), 2689. https://doi.org/10.3390/nu12092689
2. Rasinperä, H., Kuokkanen, M., Kolho, K. L., Lindahl, H., Enattah, N. S., Savilahti, E., Orpana, A., & Järvelä, I. (2005). Transcriptional downregulation of the lactase (LCT) gene during childhood. Gut, 54(11), 1660–1661. https://doi.org/10.1136/gut.2005.077404
3. Bayless, T. M., Brown, E., & Paige, D. M. (2017). Lactase Non-persistence and Lactose Intolerance. Current gastroenterology reports, 19(5), 23. https://doi.org/10.1007/s11894-017-0558-9
4. Matthew J. During, Ruian Xu, Deborah Young, Michael G. Kaplitt, Robert S. Sherwin & Paola Leone (1998). Peroral gene therapy of lactose intolerance using an adeno-associated virus vector. https://sci-hub.se/10.1038/2625
5. Chen, J., Yang, W. L., Li, G., Qian, J., Xue, J. L., Fu, S. K., & Lu, D. R. (2004). Transfection of mEpo gene to intestinal epithelium in vivo mediated by oral delivery of chitosan-DNA nanoparticles. World journal of gastroenterology, 10(1), 112–116. https://doi.org/10.3748/wjg.v10.i1.112
6. Sobh, M., Lagali, P. S., Ghiasi, M., Montroy, J., Dollin, M., Hurley, B., Leonard, B. C., Dimopoulos, I., Lafreniere, M., Fergusson, D. A., Lalu, M. M., & Tsilfidis, C. (2023). Safety and Efficacy of Adeno-Associated Viral Gene Therapy in Patients with Retinal Degeneration: A Systematic Review and Meta-Analysis. Translational vision science & technology, 12(11), 24. https://doi.org/10.1167/tvst.12.11.24
7. Maurya, S., Sarangi, P. & Jayandharan, G.R. Safety of Adeno-associated virus-based vector-mediated gene therapy—impact of vector dose. *Cancer Gene Ther* **29**, 1305–1306 (2022). https://doi.org/10.1038/s41417-021-00413-6
8. Pires AF, Marnotes NG, Rubio OD, Garcia AC, Pereira CD. Dairy By-Products: A Review on the Valorization of Whey and Second Cheese Whey. Foods. 2021; 10(5):1067. https://doi.org/10.3390/foods10051067
9. Itan, Y., Jones, B.L., Ingram, C.J. *et al.* A worldwide correlation of lactase persistence phenotype and genotypes. *BMC Evol Biol* **10**, 36 (2010). https://doi.org/10.1186/1471-2148-10-36
10. Heyer E, Brazier L, Ségurel L, et al. Lactase Persistence in Central Asia: Phenotype, Genotype, and Evolution. Human Biology. 2011 ;83(3):379-392. https://doi.org/10.3378/027.083.0304
11. Banoun H. (2023). mRNA: Vaccine or Gene Therapy? The Safety Regulatory Issues. International journal of molecular sciences, 24(13), 10514. https://doi.org/10.3390/ijms241310514
12. Dhamanti, I., Suwantika, A. A., Adlia, A., Yamani, L. N., & Yakub, F. (2023). Adverse Reactions of COVID-19 Vaccines: A Scoping Review of Observational Studies. International journal of general medicine, 16, 609–618. https://doi.org/10.2147/IJGM.S400458
13. Kohn, D.B., Chen, Y.Y. & Spencer, M.J. Successes and challenges in clinical gene therapy. *Gene Ther* 30, 738–746 (2023). https://doi.org/10.1038/s41434-023-00390-5
14. Youtube Video of gene-therapy being applied to oneself: https://www.youtube.com/watch?v=J3FcbFqSoQY&t=0s
15. Ohto, U., Usui, K., Ochi, T., Yuki, K., Satow, Y., & Shimizu, T. (2012). Crystal Structure of Human β-Galactosidase: STRUCTURAL BASIS OF GM1 GANGLIOSIDOSIS AND MORQUIO B DISEASES*. Journal of Biological Chemistry, 287(3), 1801–1812. doi: https://doi.org/10.1074/jbc.M111.293795
16. Davies, G., & Henrissat, B. (1995). Structures and mechanisms of glycosyl hydrolases. Structure, 3(9), 853–859. doi: https://doi.org/10.1016/S0969-2126(01)00220-9