IMPROVING THE ANALYSIS OF PROTEIN SECRETION INTERACTIONS IN ORGAN TISSUE
THE INCORPORATION OF GENETIC ENGINEERING IN A CLINICAL SETTING

A Thesis Prospectus
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By
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November 2, 2020

On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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The human endocrine system is responsible for facilitating the distribution of hormones through the body. These hormones are responsible for metabolism, digestion, growth and other functions. So, when the endocrine system malfunctions, it can lead to debilitating diseases and disorders like diabetes, hyperthyroid disease, and cardiovascular disease. An understanding of the endocrine system is essential to the development of optimal treatment for metabolic illnesses. Marcus M. Seldin and other microbiologists conducted an analysis of endocrine signaling amongst several tissues in mice to gain a better understanding of endocrine interactions within humans, with the speculation that the novel discoveries will be applied in other research pertaining to the downstream effects of secreted proteins (Seldin et al. 2018). The research can be improved upon in regards to the accessibility of gene expression analysis and the application of the results. The software used to conduct the bulk of the project may be further augmented to allow for a user-friendly software package of endocrine secretion analysis, which will allow for more signaling analysis with other organs. Additionally, the computational approach will acknowledge the endocrine signaling variances between sexes in humans. The technical thesis topic seeks to further explore the endocrine interactions utilizing existing data to improve the efficiency of protein secretion analysis and to apply the process to the human genome rather than mice. Computational research that seeks to advance our knowledge of the human body and the mechanisms that govern its operation are effective in the development of novel treatments. However, biomedical research involves more than just computational biological models. The improved analysis of gene expression data will allow researchers to apply this knowledge with other research fields, like genetic engineering. The combination of mapping gene expressions and gene editing may be a viable solution to many metabolic illnesses. In the STS paper, I seek to evaluate the efficacy of genetic engineering technology on metabolic diseases, and to advocate

for its adoption into the clinical setting. The Details of the thesis project progression are expressed in the figure 1 below.

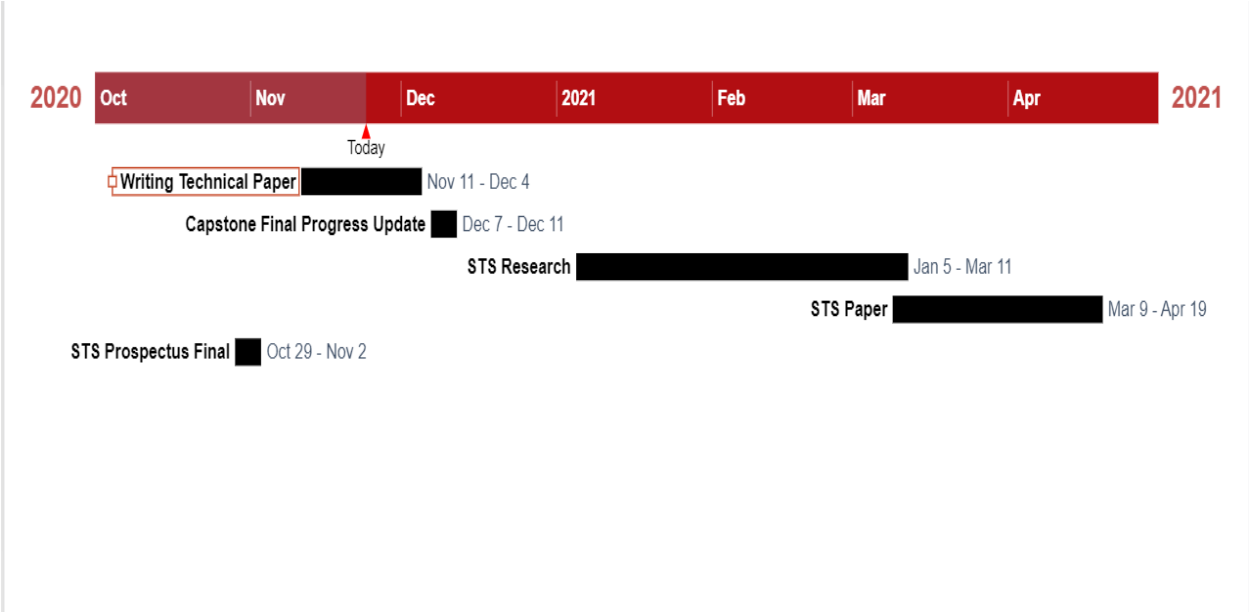

Figure 1: Progression of Thesis Project. Details the bulk processes involved and the deadlines. Conducting the technical project will occupy the majority of the project. The STS research will be conducted soon after major progress is made within the technical. (Edu Jr., 2020).

IMPROVING THE ANALYSIS OF PROTEIN SECRETION INTERACTIONS

There is a myriad of metabolic and endocrine disorders that are severely debilitating and may result in death. One of the most common lethal diseases pertaining to the endocrine system is cardiovascular disease (CVD). Nearly a quarter of U.S deaths are due to CVD, and it is the leading cause of global mortality (Khera & Sekar, 2017). Other notable metabolic diseases include obesity and type II diabetes, with 38% of adults suffering from obesity and 26 million adults diagnosed with diabetes as of 2016 (Virani Salim et al., 2020). The prevalence of these types of metabolic disorders inspired scientists to research the mechanisms of endocrine signaling between organs and to map the relationship between genetics and metabolic dysfunctionality.

One study concluded that males and females have varying gene expression levels in fat tissues, which is influential in the emergence of cardiovascular disease. Males are more likely to store excess fat tissue in their abdominal region, while women store excess adipose tissue in the thigh region (Karastergiou et al., 2012). The abdominal storage of excess fat leads to increased risk of CVD, leading to more men to suffer from this illness at an earlier age than women (Karastergiou et al., 2012). These findings spur endocrine signaling research that consider sex differences in an effort to enhance our understanding of protein-interactions in organ tissues.

Researchers inspired to make new discoveries in this particular field of study have developed computational models to map gene expressions and to analyze downstream effects on neighboring organ tissues. Some of these studies utilize gene expression data from the mouse, and apply their novel findings to the human endocrine system. This application does not completely translate despite some genetic similarities between the species (Seldin et al., 2018). Bioinformatics allows researchers to explore the inner works of protein interactions in humans *in silico* with efficiency.

The technical project incorporates the use of bioinformatics and computational models to map gene expressions in humans in order to gain a greater understanding of organ communication networks. Figure 2 displays a framework for organ tissue protein communication.

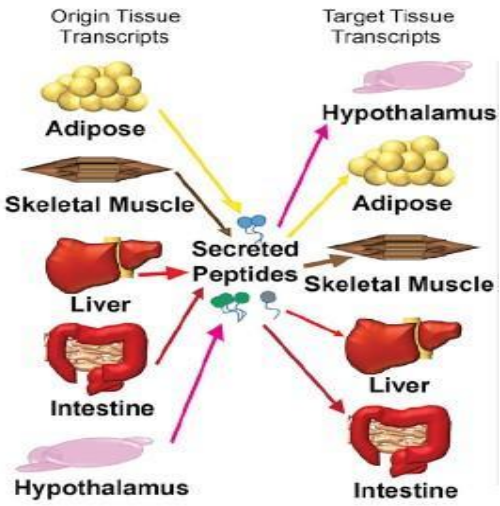

Figure 2: Organ Tissue Protein Secretions. Secreted proteins from origin organ tissue to target organs. Tissues include adipose, skeletal muscle, liver, intestinal, and hypothalamus tissue. (Seldin et al. 2018)

The potential discoveries can be applied in the clinical space, by providing physicians with the knowledge of certain biomarkers that are used to identify an emerging metabolic disease in a patient. This project also seeks to report any notable gene variances between females and males because these differences may be of significance to the comprehension of disease demographics and diagnosis. Finally, the project strives to improve the efficiency of the computational gene expression model in an effort to provide other researchers with more tools to unearth novel discoveries from human gene expression data.

To analyze gene expression data from human organs, sample RNA-Sequencing data will be acquired from a predetermined database. Sex differences between the genes will be accounted for by analyzing XIST expression data for several tissues. The data will be normalized and any novel sex differences in gene expression, will be discussed in depth within the research paper. No live experiments will be necessary since all analysis and interpretation will be performed using R software. The primary goal of this research project is to develop a user-friendly R software package to analyze gene expressions, any relevant findings pertaining to sex differences will be depicted in a scholarly article with assistance from Biomedical undergraduate students, Jonathan Blichar and Felipe Barraza. The technical process will be guided by our technical advisor, Warren Anderson, a postdoctoral fellow in the Center for Public Health Genomics at UVA. Through our research we hope to identify novel sex-specific endocrine interactions between organs. Regardless of the outcome, this project will produce a R package with a comprehensive vignette, which will be publicly available on GitHub. Public access to this package will allow many researchers to expand on gene expression studies with new tools.

THE INCORPORATION OF GENETIC ENGINEERING IN A CLINICAL SETTING

As technology advances at a rapid pace and continues to be intertwined in our society, biomedical innovations are also growing more prevalent. Biomedical research has been responsible for the development of many lifesaving technologies like cardiac pacemakers, vascular stents, biosensors, and advanced imaging techniques. There are 3 million people in the world who utilize pacemaker technology, and about 600,000 pacemakers are implanted every year (Wood & Ellenbogen, 2002). Our knowledge of the human body allows physicians and researchers to develop therapeutics to treat and cure many debilitating diseases. There are a myriad of vaccines, drugs, surgery techniques, and technological implants that operate to preserve the lives of those in need. Current methods for disease treatment are rather effective but there are still illnesses like HIV/AIDS, diabetes, and certain cancers that cannot be fully cured with the current strategies of today. Preventative measures and treatments for symptom management exist for these diseases but the medical industry still has a long way to go before we eradicate their existence. One technology that can assist in the development of therapeutics for incurable diseases is genetic engineering. Currently genetic engineering techniques have been utilized in labs to analyze and edit genes. With the emergence of the CRISPR-Cas9 gene editing technology, scientists were able to more effectively edit and insert customized RNA to edit the human genome (Pollack, 2015). This kind of technology is not currently utilized in a clinical setting but its functionality may be effective in the treatment of certain metabolic diseases. For my STS paper, I seek to evaluate the efficacy of genetic engineering technology on metabolic diseases, and to advocate for its adoption into the clinical setting.

Scientists use CRISPR-Cas9 technology to inhibit or insert customized gene sequences into a specific DNA strand. CRISPR-Cas9 accomplishes this task by using a guiding RNA

strand, provided by the researcher, to target a specific area of the DNA sequence and inserts the desired RNA strand into the DNA double-helix. The ability to effectively insert or suppress certain gene expressions is a critical technique that will greatly improve disease diagnosis and prognosis. Despite its practical uses in a lab setting, CRISPR is not ready for mass clinical use (Liu et al). Clinical trials for safety and efficacy are still being conducted before the technology is publicly available. Currently, gene editing technology is primarily used in a laboratory setting. Researchers have used this technique to alter the genetics of small animals and human cells *in vitro* for analysis of its effectiveness and functionality.

While genetic editing practices are not clinically available, researchers have made progress utilizing the technology to further understand its capabilities. One research group sought to incorporate the technology to combat HIV. University of California researcher, Yuet Kan, spearheaded a research study in 2014 which sought to use CRISPR technology to insert HIV-resistant genes within white blood cells. They found that the edited white blood cells were resistant to the virus, but further trials would have to be conducted for efficacy in an *in vivo* study (Aldhous, 2014). There have also been applications in cancer research. Oncologists at the Barts Cancer Institute published a review article detailing the use of CRISPR-Cas9 to expedite the development of oncolytic viruses, which are viruses that target cancer cells, and to evaluate its application in viral biology research (Yuan et al., 2016). Another research group at MIT used CRISPR technology to cure mice suffering from a liver disorder by correcting the mutated gene (Yin et al 2014). At the time this was one of the first research documents that proved the effectiveness of CRISPR-Cas9 to successfully cure an organism of disease. CRISPR enhanced the quality of genetic engineering research, and scientists are acknowledging its benefits with every study. Though CRISPR is not used in a clinical setting, preimplantation genetic screening

(PGS) is currently available for the public, and has been for a while now. This technique is clinically available because there is no active deletion or insertion of genes. Currently, some medical institutions offer PGS to aspiring parents, which allows the pair to select a more genetically favorable embryo from an *in vitro* collection, prior to birth. This is a rather beneficial practice because it reduces the amount of disease or disorders that a child could manifest (Brezina et al., 2013). This selection-based approach using genetic engineering techniques has granted families the ability to guarantee healthy offspring and curtail the spread of debilitating diseases.

Despite the evidence of beneficial genetic engineering techniques, certain people are hesitant about its adoption into society. A considerable amount of the resistance to genetic engineering is derived from the ethical consequences this field of medicine may invoke. These ethical topics include lack of autonomy, uniqueness, and authenticity. Michael J. Sandel, a critic of genetic modification, acknowledges the incredible benefits the technology can offer society but is cautious about its existence (2004). He believes that genetic engineering grants people "hyper-agency", the ability to remake nature and possess dominance over their natural design. He recounts in his 2004 Atlantic article, that genetic modifications will allow people to simply make better people, ones that are taller, stronger, and more intelligent. Parents' ability to create 'designer babies', genetically modified embryos, may hinder the quality of sporting competition, and make prenatal love conditional. Another critique of the adoption of genetic engineering is from theologian and Professor, Ted Peters. He writes in his book, *Playing God?: Genetic Determinism and Human Freedom,* that genetic enhancements is a practice that defies human nature and therefore God (2014). It is the mere fact that humans can alter their genetic makeup that is concerning for some individuals. This sentiment is derived from a religious perspective

but must be acknowledged because there are regulators and legislators that have the power to permit the existence of genetic engineering in a clinical setting and understanding their views may be pertinent for the adoption of the technology.

Proponents of genetic modification acknowledge the immense benefits of the practice, despite the potential risk that may come from products like ‘designer babies’. Having the ability to rid future generations from deadly disease is too great to pass up. While there isn’t much evidence that genetic engineering is the cure to all illnesses, there is enough to prove its application to the human genome will be an evolutionary contribution to medicine. Those opposed to genetic engineering must acknowledge that editing genes for not health reasons is a consequence that may arise and should not be feared. Genetic enhancements do not remove one's ability to be an autonomous individual, nor does it make them a less authentic human. Inequalities in sports already exist, genetic engineering is not altering the core nature of competition, and on the chance that it does, government regulation will preside over its application. A team of bioethicists wrote a journal article supporting the adoption of genetic modification, with the central argument being that genetic engineering is not the only governing force for human behavior, there are other factors at play that determine how people behave (Resnik and Vorhaus 2006). Genetic engineering is not like designing a robot, void of feelings and desires, it is merely an alteration on the biochemical mechanisms of an individual.

My objective is to appropriately determine the applicability of genetic engineering techniques on the treatment of metabolic disease and the inclusion of the technology in a clinical setting. To illustrate the many social entities that are involved in the adoption of a new technology, a social construction of technology (SCOT) framework will be used to map the

relationships involved in the adoption of a prominent genetic engineering technique named CRISPR-Cas9(Pinch & Bijker, 1987).

There are many stakeholders involved with a technology this powerful, and understanding the nuances that are involved with the rise of genetic modification is essential for its clinical use. The relationships between stakeholders and genetic engineering technology is depicted in Figure 3.

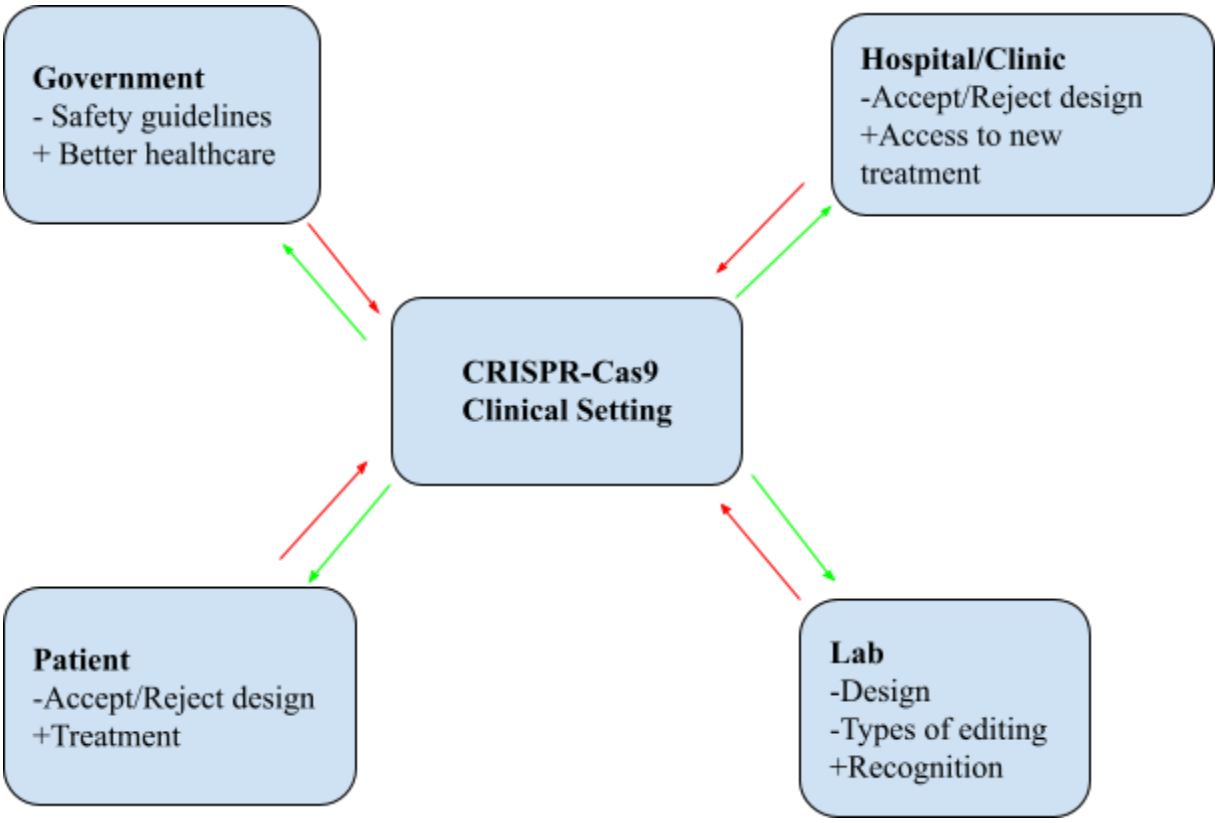

Figure 3: Social Construction of Genetic Engineering. Acknowledges the regulators, consumers, gene labs and clinics that all have stake in the incorporation of genetic engineering technology. (Edu Jr., 2020).

Regulators are heavily considered in the inclusion of CRISPR-Cas9 technology because they serve as the gatekeepers for the incorporation of its technology into society. An example of a national regulator would be the U.S. department of Health & Human Services (HHS), which

presides over the Food and Drug Administration (FDA), an institution that is responsible for regulating the majority of medical devices and products. Without national and state approval genetic modification technology will never be clinically accessible.

Not only do these entities grant access, but they also determine how the technology is used and establish safety requirements. Regulators benefit from genetic engineering techniques such as CRISPR-Cas9 because their constituents will have access to more therapeutic options for certain diseases. The labs are considered manufacturers in this sense because it is the location where gene editing is being conducted. The manufacturers are the most vital element because there is little room for error when it comes to genetic modification of human life. The 'quality' of the product must be infallible and meet all the requirements established by the regulators. The hospitals and clinics that would offer genetic modification services serve as the distributors. Without the acceptance of these entities there will be little contact between the manufacturers and the consumer. Physicians in clinics and hospitals must be confident in the gene editing techniques before offering it to their patients. Most importantly, the consumer is responsible for the development of clinically available genetic modifications because the technology does not become adopted without their trust. Also clinical trials do not exist without a consumer base that is eager to invest in the technology.

Additionally, Pacey's Triangle will be used to analyze the cultural, organizational, and technical aspects of CRISPR-Cas9. CRISPR, Cas9, cell culture, in vivo fertilization, and embryonic development. The organizational aspect would involve genetic engineering companies and labs, state and national government, and hospitals. The cultural aspect would be the most complex and nuanced due to the majority of discourse being centered around ethics. Clinically available genetic engineering would affect ethical codes, shift the perception of human

aesthetics, alter the nature of fair competition, and expose the socio-economic disparities from those who can afford it to others who cannot. A Pacey Triangle depicting the relationships between these entities is depicted in Figure 4.

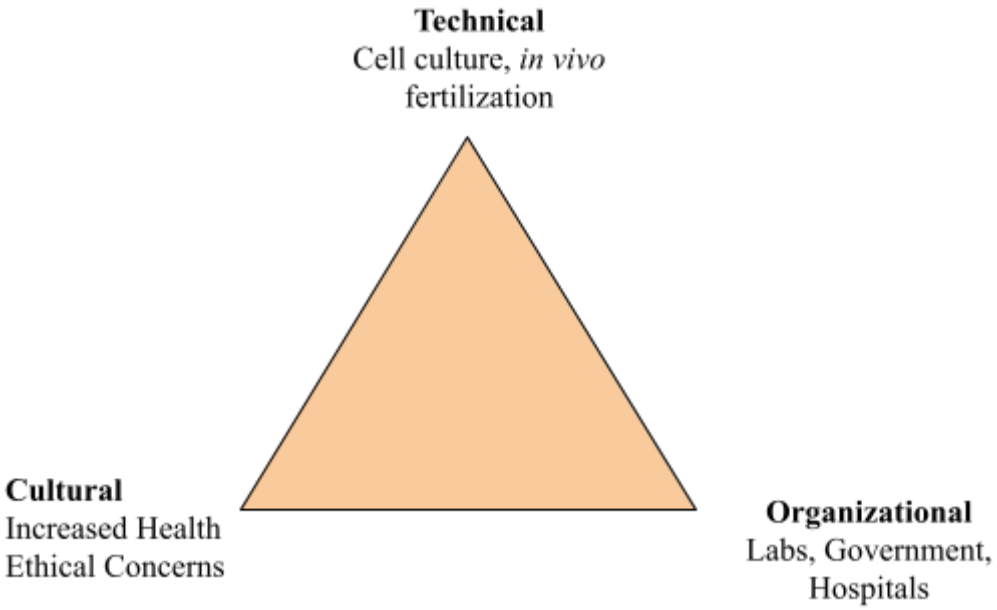

Figure 4: The Aspects Involved with Clinically Accessible Genetic Engineering. The cultural, technical, and organizational aspects of the adoption of CRISPR-Cas9 technology using Pacey's Triangle. (Edu Jr., 2020).

I anticipate that there would be a considerable amount of evidence in favor of genetic engineering techniques and the treatment of metabolic diseases. The current treatment methods have shown to alleviate symptoms of ailments like cardiovascular disease and diabetes and preventative measures are also beneficial. However, the adoption of genetic engineering practices may reduce their prevalence and decrease the likelihood of detrimental hereditary disorders. The STS research paper will be documented in a scholarly article, with relevant findings that support the thesis.

APPLICATION OF GENE MAPPING AND GENE EDITING FOR THE FUTURE

The technical portion of the project seeks to improve the usability of endocrine signaling analysis within R software, with aspirations to apply the technique for the human endocrine system and observe any discrepancies between the sexes. Ideally, the discoveries made in this report will be utilized to aid in bioinformatics genetic research. Gene expression mapping and analysis of downstream effects are critical to the understanding of certain metabolic diseases. This knowledge can be applied to genetic engineering, where scientists can augment the specific DNA sequence to successfully treat an illness. The incorporation of genetic engineering techniques like CRISPR-Cas9 may be a viable avenue to curing many debilitating metabolic illnesses, and their incorporation into the clinical setting ought to be encouraged if that were the case. To analyze the nuances of adoption of CRISPR technology, a SCOT framework will be used to identify the elements involved in its development and its more subtle implications on our culture.

WORKS CITED

Aldhous, Peter. "Gene Editing Tool Can Write HIV out of the Picture." New Scientist. Accessed October 27, 2020. https://www.newscientist.com/article/dn25698-gene-editing-tool-can-write-hiv-out-of-the-picture/.

Brezina, Paul R., Raymond W. Ke, and William H. Kutteh. "Preimplantation Genetic Screening: A Practical Guide." *Clinical Medicine Insights. Reproductive Health* 7 (February 27, 2013): 37–42. https://doi.org/10.4137/CMRH.S10852.

Edu Jr., Emmanuel E.(Unpublished 2020), School of Engineering and Applied Science, University of Virginia. Charlottesville, VA.

Karastergiou, Kalypso, Steven R. Smith, Andrew S. Greenberg, and Susan K. Fried. "Sex Differences in Human Adipose Tissues – the Biology of Pear Shape." *Biology of Sex Differences* 3, no. 1 (May 31, 2012): 13. https://doi.org/10.1186/2042-6410-3-13.

Khera, Amit V., and Sekar Kathiresan. "Genetics of Coronary Artery Disease: Discovery, Biology and Clinical Translation." *Nature Reviews Genetics* 18, no. 6 (June 2017): 331–44. https://doi.org/10.1038/nrg.2016.160.

Liu, Chang, Li Zhang, Hao Liu, and Kun Cheng. "Delivery Strategies of the CRISPR-Cas9 Gene-Editing System for Therapeutic Applications." *Journal of Controlled Release* 266 (November 28, 2017): 17–26. https://doi.org/10.1016/j.jconrel.2017.09.012.

Peters, Ted. *Playing God?: Genetic Determinism and Human Freedom*. Routledge, 2014.

Pollack, Andrew. "Jennifer Doudna, a Pioneer Who Helped Simplify Genome Editing (Published 2015)." *The New York Times*, May 11, 2015, sec. Science. https://www.nytimes.com/2015/05/12/science/jennifer-doudna-crispr-cas9-genetic-engineering.html.

Pinch, T. J., and Bijker, W. E. 1984. "The social construction of facts and artefacts: Or how the sociology of science and the sociology of technology might benefit each other." Social Studies of Science 14:399–441.

Resnik, David B., and Daniel B. Vorhaus. "Genetic Modification and Genetic Determinism." *Philosophy, Ethics, and Humanities in Medicine* 1, no. 1 (June 26, 2006): 9. https://doi.org/10.1186/1747-5341-1-9.

Sandel, Michael J. “The Case Against Perfection.” The Atlantic, April 1, 2004. https://www.theatlantic.com/magazine/archive/2004/04/the-case-against-perfection/302927/.

Seldin, Marcus M., Simon Koplev, Prashant Rajbhandari, Laurent Vergnes, Gregory M. Rosenberg, Yonghong Meng, Calvin Pan, et al. “A Strategy for Discovery of Endocrine Interactions with Application to Whole-Body Metabolism.” *Cell Metabolism* 27, no. 5 (May 2018): 1138-1155.e6. https://doi.org/10.1016/j.cmet.2018.03.015.

Virani Salim S., Alonso Alvaro, Benjamin Emelia J., Bittencourt Marcio S., Callaway Clifton W., Carson April P., Chamberlain Alanna M., et al. “Heart Disease and Stroke Statistics—2020 Update: A Report From the American Heart Association.” *Circulation* 141, no. 9 (March 3, 2020): e139–596. https://doi.org/10.1161/CIR.0000000000000757.

Wood Mark A., and Ellenbogen Kenneth A. “Cardiac Pacemakers From the Patient’s Perspective.” *Circulation* 105, no. 18 (May 7, 2002): 2136–38. https://doi.org/10.1161/01.CIR.0000016183.07898.90.

Yin, Hao, Wen Xue, Sidi Chen, Roman L. Bogorad, Eric Benedetti, Markus Grompe, Victor Koteliansky, Phillip A. Sharp, Tyler Jacks, and Daniel G. Anderson. “Genome Editing with Cas9 in Adult Mice Corrects a Disease Mutation and Phenotype.” *Nature Biotechnology* 32, no. 6 (June 2014): 551–53. https://doi.org/10.1038/nbt.2884.

Yuan, Ming, Eika Webb, Nicholas Robert Lemoine, and Yaohe Wang. “CRISPR-Cas9 as a Powerful Tool for Efficient Creation of Oncolytic Viruses.” *Viruses* 8, no. 3 (March 2016): 72. https://doi.org/10.3390/v8030072.