

# THE PROBLEM OF THE NEURODEGENERATIVE GERMLINE: AN ETHICAL RECONSIDERATION IN LIGHT OF GENETIC ENGINEERING DEVELOPMENTS

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## Abstract

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Thesis: The Problem of the Neurodegenerative Germline: An Ethical Reconsideration in Light of Genetic Engineering Developments

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In this thesis I strive to answer the question, “Is it ethically justifiable to edit out the mutant Alzheimer’s gene, and therefore the germline of, a human embryo with the CRISPR-Cas9 system?”

There are many risks of the CRISPR-Cas9 technology, including the off-target effects of mosaicism, unintended base changes, and double-stranded breaks. Along with this, using the CRISPR-Cas9 system on the germline of a human embryo violates the two major theories of bioethics as well as the principles of medical ethics. There is also a large public and religious fear about the clinical use of this technology.

Through the analysis of the science behind CRISPR-Cas9, the scientific benefits and risks of CRISPR-Cas9, and the medical ethics of its clinical use, I determined that it is not ethically justifiable to use CRISPR-Cas9 in its current state to edit the germline of a human embryo in order to remove Alzheimer’s disease. I also determined that it is not ethically justifiable to use CRISPR-Cas9 in a future, more accurate state unless multiple limitations and regulations on the technology are set.

## Biography

My name is Katherine Banner and I am a senior at The University of Texas at Austin majoring in Plan II Honors and Chemistry with a minor in Italian Language. I was born and grew up in Houston, Texas, where I attended The Kinkaid School from pre-kindergarten through twelfth grade. Throughout my college career, I have been an active member of the Camp Ozark community, where I worked for two summers, as well as the Chi Omega Sorority and the American Chemical Society. All of these organizations have helped and encouraged me to pursue my academic career and have been supportive through the thesis process, especially those who I have met through Chi Omega.

I have one older brother, Edward, who also attended The Kinkaid School and The University of Texas at Austin, and who is currently a petroleum engineer living in Dallas, Texas. Both my my parents, Ed and Valerie, are still living in Houston, where I will be moving back to this summer. I am moving back to Houston in order to attend McGovern Medical School (The University of Texas Health Science Center) and obtain my M.D. I am very excited to start this new chapter in my life at a new school and to return to Houston.

## Acknowledgements

I would first like to thank my thesis advisor, Dr. Joy Penticuff, for meeting with me so often and helping me develop, research, and edit my thesis. Without you, I would not have been able to complete this process in the manner that I wanted, and your help throughout the entire process is unmeasurable. I would also like to thank my second reader, Dr. Nancy Moran, for all of your help and input. Together, both Dr. Penticuff and Dr. Moran helped me formulate my thesis into exactly what I wanted it to be.

I would also like to thank both my parents who were constantly encouraging me to pursue a topic I loved and research and write the best I could. Thank you for all of the emails with articles relating to my topic and the constant uplifting words throughout the process. I would not have been able to make it through this year without you.

Lastly, I would like to thank Plan II for allowing me to pursue an opportunity to research and write a thesis on a topic I really love and am interested in. Not only this, but this topic was introduced to me through Plan II seminars. All of the Plan II staff has been beyond helpful, easy to communicate with, and so encouraging through the whole year and thesis process. Thank you again for everything you have provided me with through my four years of education here at UT Austin.

## I. Introduction

Modern advancement in the scientific community has been astounding, and sometimes very unexpected. We have discovered smaller particles than ever before known, bioengineered body parts for humans, developed cures and vaccinations for diseases that we never thought would be possible, and so much more. Science is leading us, as humans, down a future path that nobody expected, and a path that some may not want. Some of these new scientific discoveries, especially the new technologies involving genetic engineering, could change the human race for the good and possibly for the bad. The possibility (and success) of editing the genomes of different organisms around the world, including humans, has recently come into light, and has proven to be one of the most significant and promising technologies in the scientific and medical communities today.

However, the ethical dilemma that the new genetic engineering technologies has presented cannot be ignored. Whether these technologies should actually be used on the human population, and if so what they can and cannot be used for, is a question that can change the future of humans in innumerable ways.

There are many proposed uses of the new genetic engineering technologies, including cancer therapy and other therapies for genetic diseases. However, the ability to alter the human genome in any form raises many ethical concerns, as altering our genetic makeup can go much further than disease therapy. Altering the human genome would not only allow us to edit out mutant genes that cause disease in humans, but also to alter multiple other traits. We have already developed the technology to genetically test the genomes of human embryos (a human which is in the early stages of development and has not been born, and is derived from the zygote, which is the single cell resulting from the fertilization of an egg by a sperm) for their

genetic makeup (what genes they are positive for), and those who participate in in-vitro fertilization have the ability to take advantage of this technology. For example, parents are able to choose the sex of their child if they desire to. Any action similar to this introduces the ethical debate associated with genetic engineering: what should it be used for? There is a major fear of using the new genetic engineering technologies to perform similar actions and create “designer babies” or “genetically modified humans.”<sup>1</sup>

The genetic engineering techniques we have developed involve the precise modification of DNA and allows genes to be turned on or off, meaning that certain genes will not be physically expressed if they are turned off, and they will be expressed if they are turned on. This allows scientists to identify traits they want to remove or to insert other traits found somewhere else in an organisms’ genome through the process of editing alleles out and replacing them with non-mutant alleles. Should we alter a human embryo if we are able to genetically test and confirm that it will be born with a lethal, degenerative disease, or should we allow the human race to populate naturally? Even in the case of using genetic engineering technology on already born humans, the question of what we are able to do versus what we should do still introduces an ethical debate, as altering the germline of an adult human changes the genetic makeup of not only the adult but also any offspring of that person.

For example, some genetic mutations that lead to the development of Alzheimer’s disease are known. If we are able to test for chromosomal content of human embryos, then we are able to detect if a human embryo will be born with a genetic disease. The first use of genetic engineering technology, specifically the CRISPR-Cas9 system, on human embryos was conducted in the

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<sup>1</sup> Lander, E. S. (2015). Brave New Genome. *New England Journal of Medicine*, 373(1), 5–8. <https://doi.org/10.1056/NEJMp1506446>.

summer of 2017 in order to fix a genetic disorder in a human embryo.<sup>2</sup> Jennifer Doudna, a biologist from The University of California at Berkeley who co-discovered how to use CRISPR to edit genes, stated that “any scientist with molecular biology skills and knowledge of how to work with [embryos] is going to be able to [edit human embryos].”<sup>3</sup> We are able to genetically engineer the germline in human embryos, which can be used to treat disease in future developing humans. The concern, however, is that of Edward Lanphier, the CEO of the California biotechnology company (Sangamo Biosciences) that is using the genetic engineering technology of zinc-finger nucleases to treat HIV in adults. Lanphier believes that there is no disease rationale for germline engineering, as it is “a slippery slope toward much more unacceptable uses.”<sup>4</sup>

The consequences of genetically modifying an embryo are unknown. Currently, it is impossible for us to know if any unintended, genetic consequences will occur by genetically modifying the genome of a human embryo, and therefore it is impossible for us to know if the benefit outweighs the cost of this technology or treatment. “Any human embryo altered by CRISPR today would carry the risk that its genome had been changed in unexpected ways.”<sup>5</sup> Not only this, but modifying the genetic makeup of an adult human, and specifically the germline of an adult human, can also introduce unintended, unknown detrimental consequences to not only the adult but also any of his/her children, and the human race.

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<sup>2</sup> Park, A. (2017, August 7). U.S. Scientists Use CRISPR to Fix Genetic Disease in Human Embryos for the First Time. *Time Magazine*. Retrieved from <http://time.com/4882855/crispr-gene-editing-human-embryo/>.

<sup>3</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

<sup>4</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

<sup>5</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.



There are multiple other ethical considerations that must be taken into account when considering the human application of genetic manipulation technology besides the unintended consequences and changing a germline. When using this technology as a human treatment, there are many religious aspects to take into account, as well as geographic, social, and financial aspects.

In this thesis, I will attempt to determine whether or not it is ethically justifiable to use the CRISPR-Cas9 genetic engineering technology to edit the genetic makeup of a human embryo, and more specifically to modify the genetic makeup of the embryo's germline. The reason for this focus is because at this time, the large majority of research has been directed towards germline modification rather than somatic cell modification in human embryos. This means that my analysis of the use of the CRISPR-Cas9 genetic engineering technology deals with germline modification, which affects all future generations from the embryo upon which CRISPR-Cas9 has been applied. In order to come to a conclusion to this argument, I will analyze the scientific background of the genetic engineering technology along with any other science that relates to its clinical use, the scientific benefits and risks of the technology, and the bioethical arguments for and against the use of the CRISPR-Cas9 system on human embryos.

## **II. Scientific Background**

## Introduction

A genome is an organism's complete set of DNA, which includes all genes and hereditary material. Therefore, our genome (to an extent) determines who we are— it includes all any and all information necessary to build an organism. It determines what we look like (our phenotype), portions of our intelligence, and certain aspects of our personalities. Each gene in an organism is a specific sequence of DNA (our genotype), deoxyribonucleic acid, that codes for a certain sequence of RNA, ribonucleic acid, or a certain protein. DNA is structured as a double-helix consisting of two complementary strands made up nucleotides, which contain a phosphate group, a sugar, and a nitrogenous base. There are four different nitrogenous bases in DNA: adenine (A), cytosine (C), guanine (G), and thymine (T), and RNA has the same four except that uracil (U) is substituted for thymine. In the complementary strands, adenine pairs with thymine and cytosine pairs with guanine. Each DNA sequence that codes for a gene is converted into a protein through two different processes that make up the central dogma of genetics. DNA is unwound and replicated, and then converted into RNA (which is single-stranded rather than double-stranded) through a process called transcription, and then the RNA is translated into the corresponding protein through a process called translation. These processes are continuously occurring in our bodies to maintain every one of our bodily functions.

Genetic engineering has the ability to change our genome by manipulating our genes through biotechnology, which is technology that utilizes living organisms to develop a desired product. "Genome engineering technology offers unparalleled potential for modifying human and nonhuman genomes. In humans, it holds the promise of curing genetic disease."<sup>6</sup> These

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<sup>6</sup> Baltimore, D., Berg, P., Botchan, M., Carroll, D., Charo, R. A., Church, G., ... Yamamoto, K. R.

genetic engineering techniques involve the precise modification of DNA through introducing mutations and allows for genes to be turned on or off. However, through altering our genetic makeup we have the ability to alter our germline, which is what we pass on to our future offspring, what our offspring pass on to their offspring, and so on. Our germline consists of germ cells that eventually produce sperm and eggs, and therefore contains *all* of the genetic information to be passed on.

### The CRISPR-Cas9 Genetic Engineering System

One specific technology recently introduced to the scientific community is Clustered Regularly Interspaced Short Palindromic Repeats- associated protein-9 nuclease (CRISPR-Cas9), which is used to introduce targeted mutations (insertions, deletions, and substitutions) into specific gene sequences.<sup>7, 8</sup> A palindromic sequence of DNA or RNA is a sequence in which the sequence (reading from left to right, or 5' to 3') on the template strand matches the sequence (reading right to left, also 5' to 3') on the complementary strand. Repeats of sequences like these are involved in the CRISPR-Cas9 system. Insertions are genetic mutations in which a single base or group of bases are inserted into a genetic sequence, deletions are mutations in which a single base or group of bases are deleted from a genetic sequence, and a substitution is a mutation in which one base is substituted for another. The CRISPR-Cas9 system has many current

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(2015). A prudent path forward for genomic engineering and germline gene modification. *Science*, 348(6230), 36–38. <https://doi.org/10.1126/science.aab1028>.

<sup>7</sup> Liang, P., Xu, Y., Zhang, X., Ding, C., Huang, R., Zhang, Z., ... Huang, J. (2015). CRISPR/Cas9-mediated gene editing in human trippronuclear zygotes. *Protein & Cell*, 6(5), 363–372. <https://doi.org/10.1007/s13238-015-0153-5>.

<sup>8</sup> Peng, Y. (2016). The morality and ethics governing CRISPR–Cas9 patents in China. *Nature Biotechnology*, 34(6), 616–618. <https://doi.org/10.1038/nbt.3590>.

applications. Since the CRISPR-Cas9 system has the ability to target and induce mutations in one or two alleles, it can mimic heterozygous or homozygous knockout of a specific gene.<sup>9</sup> Therefore, this technology is essentially able to make certain genes inoperative, and in the case of gene therapy, it makes mutant, disease-causing alleles inoperative through gene editing. The CRISPR-Cas9 genetic engineering technology can correct genetic defects through introduction of new DNA sequences, change DNA sequences in pluripotent embryonic stem cells to culture certain specific differentiated tissues, and change germline DNA by altering the DNA in the nuclei of reproductive cells. Through modifying the genome of fertilized animal eggs and embryos, the CRISPR-Cas9 system has the ability to alter the genetic makeup of every differentiated cell in an organism, therefore confirming that the changes will be passed on to the offspring of the organism.<sup>10</sup>

The CRISPR-Cas9 is a very new technology. CRISPRs were first investigated in the 2000s and were later discovered in many bacteria and archaea. A few years later, in 2005, CRISPR was discovered to be derived from plasmid and viral origins, and it was found that CRISPR loci were transcribed. Combined with the observation that the Cas gene encodes proteins with nuclease and helicase domains, it was proposed that CRISPR-Cas could be a defense mechanism that had RNA memory signatures of past invasions. In 2007, the CRISPR-Cas-mediated adaptive immunity was proved in *Streptococcus thermophilus*, and just a year later mature CRISPR RNAs (crRNAs) were illustrated to be guides in complexes with Cas proteins

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<sup>9</sup> Tu, Z., Yang, W., Yan, S., Guo, X., & Li, X.-J. (2015). CRISPR/Cas9: a powerful genetic engineering tool for establishing large animal models of neurodegenerative diseases. *Molecular Neurodegeneration*, 10(1). <https://doi.org/10.1186/s13024-015-0031-x>.

<sup>10</sup> Baltimore, D., Berg, P., Botchan, M., Carroll, D., Charo, R. A., Church, G., ... Yamamoto, K. R. (2015). A prudent path forward for genomic engineering and germline gene modification. *Science*, 348(6230), 36–38. <https://doi.org/10.1126/science.aab1028>.

and the DNA-targeting activity of CRISPR was determined.<sup>11</sup>

The CRISPR-Cas9 system (see Figure 1)<sup>12</sup> is derived from the bacteria *Streptococcus pyogenes* SF370, and uses specific RNA molecules within an RNA duplex (tracrRNA:crRNA) from this bacteria that have the ability to recognize human DNA sequences.<sup>13</sup> It is derived from type II CRISPR-Cas systems in bacteria that provide bacteria with adaptive immunity to certain viruses and plasmids. The

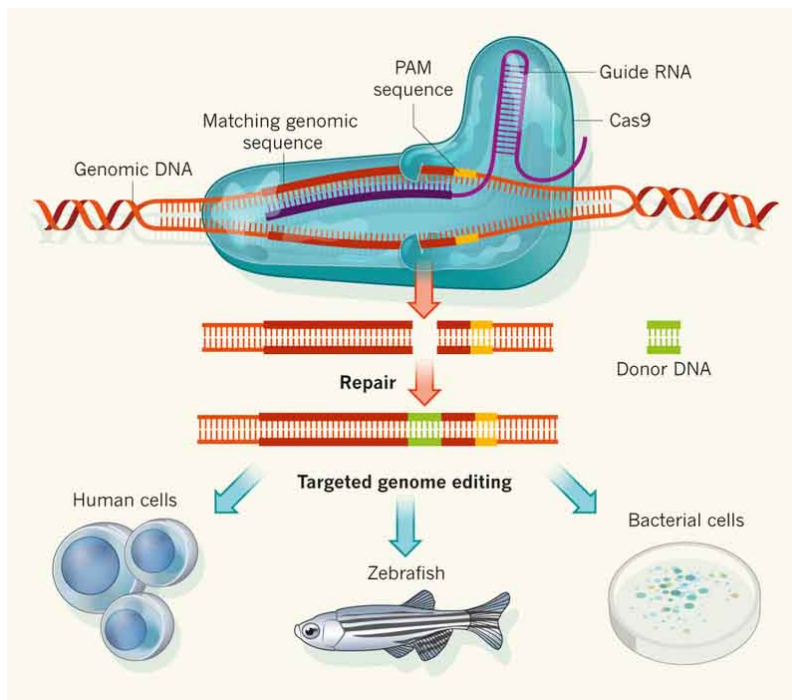


Figure 1: Simple Diagram of the CRISPR-Cas9 System

bacterial RNA is used as a guide that leads the endonuclease, the CRISPR-associated protein Cas9, to the targeted, matching location in the human genome in order to form base pairs.<sup>14, 15</sup> A nuclease is a type of enzyme (biological catalyst that can accelerate chemical reactions) that is

<sup>11</sup> Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, 346(6213), 1258096–1258096. <https://doi.org/10.1126/science.1258096>.

<sup>12</sup> OriGene Technologies, Inc. (2018). CRISPR-Cas9, Gene Editing Tool. Retrieved from [www.origene.com/products/gene-expression/crispr-cas9](http://www.origene.com/products/gene-expression/crispr-cas9).

<sup>13</sup> Liang, P., Xu, Y., Zhang, X., Ding, C., Huang, R., Zhang, Z., ... Huang, J. (2015). CRISPR/Cas9-mediated gene editing in human trippronuclear zygotes. *Protein & Cell*, 6(5), 363–372. <https://doi.org/10.1007/s13238-015-0153-5>.

<sup>14</sup> Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, 346(6213), 1258096–1258096. <https://doi.org/10.1126/science.1258096>.

<sup>15</sup> Lanphier, E., Urnov, F., Haecker, S. E., Werner, M., & Smolenski, J. (2015). Don't edit the human germ line. *Nature*, 519(7544), 410–411. <https://doi.org/10.1038/519410a>.

able to cleave nucleic acids, the group of biological molecules that includes DNA and RNA. The CRISPR-Cas9 technology is simpler than others as it does not rely on the successful engineering of certain proteins that can bind to certain, specific engineering sequences, but rather just the correct pairing of RNA and DNA.<sup>16</sup> Before CRISPR, it was not possible to precisely change the DNA of primates, and now we are able to do this easily and efficiently.<sup>17</sup>

More specifically, the formation of base pairs with target DNA sequences that contain trinucleotide protospacer adjacent motifs (PAM) allows the CRISPR-associated protein Cas9 to cleave the target DNA, introducing a site-specific double-strand break in the DNA. A double-strand break in DNA is when both strands of a DNA duplex are cleaved, and if undesired are very detrimental and can be potentially lethal. The double-strand breaks in the target DNA (which is usually a sequence about twenty-three nucleotides long) are repaired by non-homologous end-joining or homologous recombination directed repair.<sup>18,19</sup>

The specific, dual tracrRNA:crRNA was engineered in this technology as a single guide RNA (sgRNA), and it has two very necessary features for successful use of the technology: a specific sequence at the 5' end of the RNA that determines the DNA target site in the organism and a duplex RNA structure at the 3' end of the RNA that binds to the protein Cas9. Through

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<sup>16</sup> Lanphier, E., Urnov, F., Haecker, S. E., Werner, M., & Smolenski, J. (2015). Don't edit the human germ line. *Nature*, *519*(7544), 410–411. <https://doi.org/10.1038/519410a>.

<sup>17</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

<sup>18</sup> Liang, P., Xu, Y., Zhang, X., Ding, C., Huang, R., Zhang, Z., ... Huang, J. (2015). CRISPR/Cas9-mediated gene editing in human trippronuclear zygotes. *Protein & Cell*, *6*(5), 363–372. <https://doi.org/10.1007/s13238-015-0153-5>.

<sup>19</sup> Tu, Z., Yang, W., Yan, S., Guo, X., & Li, X.-J. (2015). CRISPR/Cas9: a powerful genetic engineering tool for establishing large animal models of neurodegenerative diseases. *Molecular Neurodegeneration*, *10*(1).

these two features, the CRISPR-Cas9 technology can target any desired DNA sequence.<sup>20</sup> Once the Cas9 protein is expressed, it forms a riboprotein complex with the sgRNA through interactions between what is called the scaffold domain of the sgRNA and positively-charged grooves of amino acids on the Cas9 protein that are exposed to the surface. This causes a conformational change of Cas9 into the active form that allows it to bind to DNA with the PAM, and the spacer sequence of the sgRNA is left free so it can also interact with the DNA.<sup>21</sup> After association with the double-stranded DNA, Cas9 undergoes further conformational change, creating a channel between the two structural lobes of the Cas9 protein that can bind to the RNA-DNA hybrid and to the stacked dual-RNA structure of the sgRNA. The hinge and bridge between these two structural lobes of Cas9, an arginine-rich  $\alpha$ -helix, plays a central role in binding the sgRNA-target DNA hybrid.<sup>22</sup>

### Other Technologies Similar to the CRISPR-Cas9 System

Along with the already observed success of modifying human embryos, the CRISPR-Cas9 system has the benefit of being developed after older, less-successful technologies. The CRISPR system was developed to be a more precise and successful technology with potential

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<sup>20</sup> Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, 346(6213), 1258096–1258096. <https://doi.org/10.1126/science.1258096>.

<sup>21</sup> CRISPR/Cas9 guide. AddGene. <https://www.addgene.org/crispr/guide/>.

<sup>22</sup> Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, 346(6213), 1258096–1258096. <https://doi.org/10.1126/science.1258096>.

use on humans. Before CRISPR, there were (and still are) three other classes of engineering nucleases used for the purpose of gene editing: zinc finger nucleases, transcription activator-like nucleases (TALENs), and engineered meganucleases.<sup>23</sup> The CRISPR-Cas9 system is the only one that utilizes a RNA-guided system, and it is, by far, the most specific of these gene-editing technologies. Compared to the other, similar genetic engineering technologies, CRISPR-Cas9 is much more advanced in the fact that it is the only one that has the ability to make extremely specific edits to the genome that could consist of just one base pair, and then repair the break after.

### Alzheimer's Disease

The CRISPR-Cas9 system has already been proposed for treatment for genetic disease and altering human embryos. In the case of certain genetic diseases in humans, more than 50,000 genetic mutations that are linked to disease are caused by one base change, and most commonly that DNA change is from a G-C pair to T-A.<sup>24</sup>

Alzheimer's disease is one neurodegenerative disease in which the genetic components are known, and gene therapy is a viable option for Alzheimer's alleviation. It is a brain disorder with symptoms of progressive dementia, loss of memory and thinking ability, and loss of ability to do some normal day-day actions (and it worsens over time).<sup>25</sup> In Alzheimer's disease, nerve

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<sup>23</sup> Meštrović, T., M.D.,PhD. (2016, January 13). How Does CRISPR Compare to Other Gene-Editing Techniques? Retrieved from <https://www.news-medical.net/life-sciences/How-Does-CRISPR-Compare-to-Other-Gene-Editing-Techniques.aspx>.

<sup>24</sup> Belluz, J., & Irfan, U. (2017, October 25). Two new CRISPR tools overcome the scariest parts of gene editing. Retrieved from <https://www.vox.com/2017/10/25/16527370/crispr-gene-editing-harvard-mit-broad>.

<sup>25</sup> McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group\* under the



cells deteriorate and therefore brain matter decreases (the cortex of the brain and the hippocampus are significantly reduced). About 5.5 million Americans currently have Alzheimer's disease, and it is estimated that up to 16 million Americans will have this disease in the year 2050.<sup>26</sup> There is no single cause of Alzheimer's disease but rather it is a combination of several different gene mutations and related resultant problems. It is primarily caused by two factors:  $\beta$ -amyloid plaques and neurofibrillary tangles.  $\beta$ -amyloid plaques are largely caused by deposits of 39-43 amino acid-long  $\beta$ -amyloid peptide (which is from a larger  $\beta$ -amyloid precursor protein). Neurofibrillary tangles are aggregates of hyperphosphorylated tau protein.<sup>27</sup>

The human tau gene is located on chromosome 17, and the tau protein (a phosphoprotein) stimulates tubulin assembly into microtubules in the brain— it is the major microtubule associated protein (MAP) of mature neurons.<sup>28</sup> Tau is modified post-translationally, and the phosphorylation of tau negatively regulates its activity in the production of microtubule assembly. The hyperphosphorylation on tau protein its C-terminus end causes the aggregation of the protein, leading to the formation of neurofibrillary tangles. One possible target for neurofibrillary tangle alleviation is to genetically target GSK-3 $\beta$  (glycogen-synthase kinase-3 $\beta$ ), a kinase that is involved in the pathogenesis of Alzheimer's disease (a kinase is an enzyme that chemically modifies other proteins by adding phosphate groups). The inhibition of GSK-3 $\beta$

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auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*, 34(7), 939–939. <https://doi.org/10.1212/WNL.34.7.939>.

<sup>26</sup> American Alzheimer's Association. (2018). Alzheimer's Association. <https://www.alz.org/>.

<sup>27</sup> Shoghi-Jadid, K., Small, G. W., Agdeppa, E. D., Kepe, V., Ercoli, L. M., Siddarth, P., ... Barrio, J. R. (2002). Localization of Neurofibrillary Tangles and Beta-Amyloid Plaques in the Brains of Living Patients With Alzheimer Disease. *The American Journal of Geriatric Psychiatry*, 10(1), 24–35. <https://doi.org/10.1097/00019442-200201000-00004>.

<sup>28</sup> Iqbal, K., Liu, F., Gong, C.-X., & Grundke-Iqbal, I. (2010). Tau in Alzheimer disease and related tauopathies. *Current Alzheimer Research*, 7(8), 656–664.

through genetic means has shown to reverse hyperphosphorylation of the tau protein in mice, and it is known that the human body tolerates inhibition of GSK-3 $\beta$  well.<sup>29</sup> Targeting the mutated genes of tau kinases and tau proteins could lead to alleviation of neurofibrillary tangles in Alzheimer's patients and therefore could alleviate symptoms of Alzheimer's patients. The *MAPT* (microtubule associated protein tau) gene is the gene that encodes for tau proteins. Mutation of the *MAPT* gene results in the accumulation and hyperphosphorylation of tau proteins, and therefore genetically targeting the mutated form of this gene would reduce the instance of hyperphosphorylated tau.<sup>30</sup>

The other major player of Alzheimer's disease is accumulation of  $\beta$ -amyloid plaques.  $\beta$ -amyloid plaques are a result of fragments of an amyloid precursor protein (APP) that harden in the brain and become insoluble (and therefore are unable to be eliminated).<sup>31</sup> One of the main genetic factors in Alzheimer's disease is apolipoprotein  $\epsilon$ -4 (APOE-4), which is a precursor to the formation of  $\beta$ -amyloid plaques.<sup>32,33</sup> Apolipoprotein E is involved with injury repair in the brain. It regulates lipid balance through the mediation of lipid transport into and out of the brain,

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<sup>29</sup> Gong, C.-X., & Iqbal, K. (2008). Hyperphosphorylation of Microtubule-Associated Protein Tau: A Promising Therapeutic Target for Alzheimer Disease. *Current Medicinal Chemistry*, 15(23), 2321–2328. <https://doi.org/10.2174/092986708785909111>.

<sup>30</sup> Iqbal, K., Liu, F., Gong, C.-X., & Grundke-Iqbal, I. (2010). Tau in Alzheimer disease and related tauopathies. *Current Alzheimer Research*, 7(8), 656–664.

<sup>31</sup> Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., ... Cole, G. (1996). Correlative memory deficits, A $\beta$  elevation, and amyloid plaques in transgenic mice. *Science (New York, N.Y.)*, 274(5284), 99–102.

<sup>32</sup> Gong, C.-X., & Iqbal, K. (2008). Hyperphosphorylation of Microtubule-Associated Protein Tau: A Promising Therapeutic Target for Alzheimer Disease. *Current Medicinal Chemistry*, 15(23), 2321–2328. <https://doi.org/10.2174/092986708785909111>.

<sup>33</sup> Dacks, P., PhD. (2016, November 16). What APOE Means for Your Health. Retrieved from <https://www.alzdiscovery.org/cognitive-vitality/blog/what-apoe-means-for-your-health>.

which in turn helps with repair. There are three different isoforms of APOE, all of which bind to lipids, certain receptors, and  $\beta$ -amyloid proteins, but in different manners.<sup>34</sup> It is specifically the  $\epsilon$ -4 allele that increases likelihood of Alzheimer's disease, and not the other two isoforms of APOE. "Currently, the best arguments [for human implementation of genetic engineering technology] might be for eliminating the  $\epsilon$ 4 variant at the *APOE* gene (which increases risk for Alzheimer's disease and cardiovascular disease)."<sup>35</sup>

### Conclusions

The science behind both the CRISPR-Cas9 system and Alzheimer's disease, when considered in conjunction, introduces a discussion of them being used together for medical benefit. Knowing certain genetic players in Alzheimer's disease allows us to consider using the CRISPR-Cas9 system as possibly gene therapy to target the mutations that eventually lead to onset and progression of Alzheimer's disease. The genetic component of Alzheimer's disease is one that can be examined and easily tested for in human embryos, although it would be very difficult to genetically repair brain cells in developed, adult individuals. By genetically modifying embryos that have been tested to have the mutations leading to Alzheimer's may, if successful, prove to be a viable therapy for those who would not have other viable options in life. There has been more than one human trial showing that the CRISPR-Cas9 system can be used in humans, and other trials are leading towards and testing human embryos. However, although possibly successful with further development, there are multiple other aspects of this treatment

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<sup>34</sup> Liu, C.-C., Liu, C.-C., Kanekiyo, T., Xu, H., & Bu, G. (2013). Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nature Reviews. Neurology*, 9(2), 106–118. <https://doi.org/10.1038/nrneurol.2012.263>.

<sup>35</sup> Lander, E. S. (2015). Brave New Genome. *New England Journal of Medicine*, 373(1), 5–8. <https://doi.org/10.1056/NEJMp1506446>.

that need to be brought into light in order to determine if this technology should ever actually be implemented in humans.

### **III. Scientific and Medical Benefits and Risks**

#### Introduction

Along with the many ethical concerns regarding the general field of genetic engineering and of the CRISPR-Cas9 technology itself that will be discussed later on in the thesis, there are also many benefits and risks from the scientific standpoint that must be considered. Whether or

not the scientific technology itself is safe enough to use on humans is especially important to consider before analyzing the ethical concerns human use— the CRISPR-Cas9 genetic engineering system must have minimal side effects and a very high accuracy rate in order to be considered acceptable to use on humans in a clinical setting. There have been other technologies similar to CRISPR which make CRISPR an impressive comparison. It is the only technology in this field that has introduced the option of clinically alleviating and curing human genetic diseases, especially those caused by a single base pair or just a couple base changes. The use of the CRISPR-Cas9 technology on humans would also allow further clinical research on inherited diseases in humans. However, there are also many off-target, unexpected risks of using CRISPR like unpredicted base changes, the creation of mosaic mutations, and double-stranded break errors. There have been multiple trials using CRISPR-Cas9 to edit non-human DNA, but most recently many that have edited human embryos, some viable and some non-viable. The results of each trial, as discussed below, are very similar yet also very different from one another in terms of the consequences and just how they were carried out experimentally. However, each trial also shows potential scientific benefits and risks of using the CRISPR-Cas9 system on human embryos.

#### Genetic Engineering Trials: Benefits

The first attempt at genetically modifying a human embryo in the US occurred in summer of 2017 by a research team in Portland, Oregon at Oregon Health and Science University. This research team, led by Shoukhrat Mitalipov, used the CRISPR system to edit multiple one-cell human embryos. According to Mitalipov, the inherited defective genes were edited efficiently, and most importantly, edited safely. This means that there were no observed detrimental (and

unexpected) off-target effects in the embryos in the trial. After performing the trial, his team concluded that with CRISPR, it is possible to avoid off-target, unexpected effects. However, none of the embryos developed further than a few days, so the later possible side effects could not be examined.<sup>36</sup>

Not only was editing human embryos attempted in the United States, but it was also researched and attempted by researchers in China. The Chinese researchers at Sun Yat-sen University used CRISPR-Cas9 to alter mutant HBB gene that causes the  $\beta$ -thalassemia human disease. In each of their trials, non-viable human embryos were used. Although they were able to successfully edit out the mutant HBB gene in one of their trials, they reported multiple off-target effects and mosaicism, both which mean that some un-targeted DNA was edited.<sup>37</sup> In both of the above cases, the germline of the human embryo was altered, whether the target gene was completely or only partially edited.

One reason Mitalipov's group overcame the off-target effects and mosaicism was timing. Although possibly inapplicable to viable human embryos going through the in-vitro fertilization process, his group injected the CRISPR-Cas9 system into eggs at the same time they were fertilized with sperm (so at the moment of fertilization and zygote formation). He observed that compared to other technologies, it is possible to overcome the side effects for a truly beneficial outcome, even though the embryos in question were only observed for a few days. One of the largest risks of using the CRISPR-Cas9 technology to alter the human genome is the possible mosaic outcome, so the ability to overcome this issue is a large discovery in the field. However,

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<sup>36</sup> Connor, S. (2017). First Human Embryos Edited in U.S. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/608350/first-human-embryos-edited-in-us/>.

<sup>37</sup> Brown, H. (2015, April 26). World's First Genetically Modified Human Embryo Raises Ethical Concerns. <http://theconversation.com/worlds-first-genetically-modified-human-embryo-raises-ethical-concerns-40766>.

as stated, the embryos were observed for only a few days so the possible mosaic phenotype may have not been observed (although seemingly not visible in the genotype). Mitalipov was also the scientist to introduce the world's first cloned monkey, marking him as a leader in the field of genetics.<sup>38</sup>

Without the accidental, unpredicted consequences, the CRISPR-Cas9 technology would be beneficial in a clinical setting. The elimination of off-target effects would be one step closer to allowing the use of the CRISPR-Cas9 technology in a clinical setting, and therefore one step closer to the use of CRISPR-Cas9 for disease prevention, elimination, and alleviation. If clinically used on viable human embryos to go through in-vitro fertilization, it is possible that we could alleviate or even eliminate degenerative diseases that greatly affect the human population. With these recent discoveries in the CRISPR-Cas9 technology and the more general field of genetic engineering as a whole, it is necessary to further research the inherited diseases that CRISPR could be used for. This further research on inherited diseases could not only improve the outcome of genetically editing mutant genes, but also would improve the alleviation and curing of the diseases that are already present in adult humans.

As the specific base changes that cause many genetic diseases are known (see chapter two), scientist David Liu from Harvard developed the enzyme adenosine deaminase and attached it to the CRISPR-Cas9 system, which led to a very efficient process with very few off-target effects. In 28 percent of the cells tested by his research group, a mutation was successfully reversed with no unintended modifications. In the scientific rather than ethical sense, this

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<sup>38</sup> Connor, S. (2017). First Human Embryos Edited in U.S. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/608350/first-human-embryos-edited-in-us/>.

experiment leads CRISPR in a direction for efficient use in permanent modification of mutations for therapeutic reasons.<sup>39</sup>

### Genetic Engineering Trials: Risks

Although there are clear scientific benefits of using CRISPR and other genetic engineering technologies, there are many more scientific risks than benefits that may outweigh the benefits in the end. The unknown and known complications of the CRISPR-Cas9 system have led high-profile officials, like the US Intelligence agency, to label this gene editing technology a “weapon of mass destruction.”<sup>40</sup> Officials are worried that it could be used to make viruses and plagues that can wipe out crops or severely alter human health. Although this could be a large threat to the human population, the US Intelligence Agency was also extremely worried about unregulated use of the technology, and how that would transfer over to heritable genes. For example, unregulated use of the CRISPR-Cas9 technology on humans could allow for the editing of physical traits in embryos. The more a certain physical trait was edited out and a different one edited in, there is a possibility of eliminating certain physical, heritable traits.

Although CRISPR has been used to modify human embryos in successful trials, the unknown side complications like unpredicted base changes, mosaicism, and double-stranded breaks could mark CRISPR to be unsuccessful in its current state. The large possibility of off-target effects means that there is a large possibility of unknown and unexpected detrimental and

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<sup>39</sup> Belluz, J., & Irfan, U. (2017, October 25). Two new CRISPR tools overcome the scariest parts of gene editing. Retrieved from <https://www.vox.com/2017/10/25/16527370/crispr-gene-editing-harvard-mit-broad>.

<sup>40</sup> Regalado, A. (2016). Top U.S. Intelligence Official Calls Gene Editing a WMD Threat. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/600774/top-us-intelligence-official-calls-gene-editing-a-wmd-threat/>.



maybe fatal complications, as the system could introduce other mutations that cause terrible illnesses and malfunctions. The trials that proved CRISPR successful in the modification of human embryos only observed the embryos for a few days prior to the experiment, which is not enough time to confirm that unexpected off-target effects would not take effect in the future of the embryo. There has not been a trial where a human embryo is viable to develop as a normal functioning human being (as it has not been approved yet), so the listed consequences are still unpredictable.

### Possible Unintentional Risks of the CRISPR-Cas9 System Explained

#### Unintended Base Changes

The CRISPR-Cas9 technology, as stated previously, can edit out unwanted target genes and replace them with the desired gene. However, when changing the bases of the genome, more than just the target gene may be edited. Just a single base insertion, where one base is entered into the base sequence, or a single base deletion, where one base is deleted from the base sequence, can cause translation errors that affect a large portion of the genome and possibly change the target gene to an unwanted product. This unpredicted translation error (the processes of transitioning RNA to proteins in the body) in the heritable portion of the genome would cause permanent, possibly detrimental or even fatal consequences later in life. All edits made by the CRISPR-Cas9 system are permanent. It can also only be used where cells are actively dividing, as the Cas9 machinery is linked to cell division.<sup>41</sup> The unintended, off-target gene edits caused by CRISPR is one of the largest and most scientifically and ethically concerning aspect of

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<sup>41</sup> Belluz, J., & Irfan, U. (2017, October 25). Two new CRISPR tools overcome the scariest parts of gene editing. Retrieved from <https://www.vox.com/2017/10/25/16527370/crispr-gene-editing-harvard-mit-broad>.

the technology. For example, one CRISPR-Cas9 trial on viable embryos in China was attempting to correct the beta 41-42 mutation, which causes beta-thalassemia, but instead of fixing it another mutation was introduced instead.<sup>42</sup> However, there has been one recent modification to CRISPR, where the Cas13 protein is used instead of Cas9, so that edits can be made to transient genetic material and the changes are reversible. An alteration of the CRISPR-Cas9 system similar to this would allow changes to be reversible, and any detrimental effects could be reversed if necessary. Therefore, it could fix certain genetic mutations without affecting the genome.<sup>43</sup>

### Mosaicism

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<sup>42</sup> Le Page, M. (2017, March 9). First Results of CRISPR Gene Editing of Normal Embryos Released. Retrieved from <https://www.newscientist.com/article/2123973-first-results-of-crispr-gene-editing-of-normal-embryos-released/>.

<sup>43</sup> Belluz, J., & Irfan, U. (2017, October 25). Two new CRISPR tools overcome the scariest parts of gene editing. Retrieved from <https://www.vox.com/2017/10/25/16527370/crispr-gene-editing-harvard-mit-broad>.

Besides unintended base changes, another risk of the CRISPR gene editing technology is mosaicism. One Chinese research team at the Third Affiliated Hospital of Guangzhou Medical University carried out a CRISPR trial on viable human

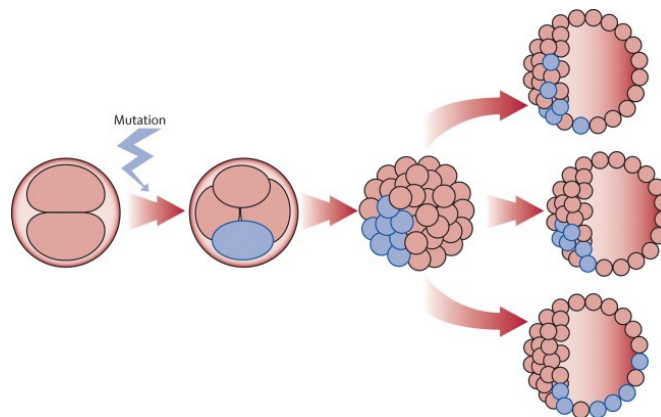


Figure 2: Mosaicism in 4-stage Cell Embryo

embryos with a heritable disease that

were donated by patients of in-vitro fertilization, and actually had more success than their trials on non-viable human embryos. This research team was the same team that carried out the beta 41-42 mutation trial in China. However, the largest consequence they encountered with the CRISPR technology was the creation of a mosaic embryo. In the cases of these embryos, the G6PD gene (which was the target gene), was completely turned off instead of edited.

“[Mosaicism] would need to be solved before the methods could be used clinically to correct a disease” said Robin Lovell-Badge of the Francis Crick institute in London.<sup>44</sup> Mosaic embryos are embryos that have a mixture of edited and non-edited cells (see Figure 2).<sup>45</sup> With only partial editing of the embryo, it is still possible that the child would still grow up with the disease in question.<sup>46</sup> This consequence occurs when the Cas9 mRNA is translated at a later developmental

<sup>44</sup> Le Page, M. (2017, March 9). First Results of CRISPR Gene Editing of Normal Embryos Released. Retrieved from <https://www.newscientist.com/article/2123973-first-results-of-crispr-gene-editing-of-normal-embryos-released/>.

<sup>45</sup> Memorial Şişli IVF & Genetics Center. Preimplantation Genetic Diagnosis by Next Generation Sequencing (NGS). Retrieved from <http://www.tupbebek-genetik.com/en/genetics/next-generation-sequencing-ngs/>.

<sup>46</sup> Le Page, M. (2017, March 15). Mosaic Problem Stands in the Way of Gene Editing Embryos.

stage than the sg RNA, so various different alleles result.<sup>47</sup> One possible remedy for this problem would be to edit the eggs and sperm separately before fertilization, but that has not been attempted yet. Another hypothesis in the reduction of mosaic mutations is the promotion of degradation of the Cas9 protein. The prolonged expression of Cas9 in embryos could contribute to the creation of mosaic DNA mutations, so tagging Cas9 with ubiquitin in order to degrade it faster could reduce the mosaic mutations. It has been proven that tagging Cas9 with ubiquitin reduces its half-life and reduces the number of mosaic mutations. However, this has only been tested on monkeys and not human embryos.<sup>48</sup> With mosaic mutations, “only about half the embryos will lead to live births, and of those that do, many could contain a mixture of cells with edited DNA and without,” according to Guoping Feng of MIT’s McGovern Institute for Brain Research.<sup>49</sup>

### Double-Stranded Breaks

One last large complication of the CRISPR system is the induction of unwanted, extra double-stranded breaks. CRISPR induces a double-stranded break in order to edit the DNA. Normally the break is rejoined by non-homologous end joining or homology-directed repair mechanisms, but when an extra break is induced that was unintended it may not be repaired by the specific mechanisms. There also may be an issue that the intended double-stranded break is

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Retrieved from <https://www.newscientist.com/article/mg23331174-400-mosaic-problem-stands-in-the-way-of-gene-editing-embryos/>.

<sup>47</sup> The Jackson Laboratory. (2018). Mosaicism. Retrieved from <https://www.jax.org/jax-mice-and-services/model-generation-services/crispr-cas9/mosaicism#>.

<sup>48</sup> Tu, Z., Yang, W., Yan, S., Yin, A., Gao, J., Liu, X., ... Li, X.-J. (2017). Promoting Cas9 degradation reduces mosaic mutations in non-human primate embryos. *Scientific Reports*, 7, 42081. <https://doi.org/10.1038/srep42081>.

<sup>49</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

never made at all. For example, studies have shown that a single mis-match in the sgRNA and target strands of DNA can prevent the Cas9-mediated cleavage. There are currently methods to predict off-target breaks when Cas9 is used in vivo, but not in vitro and the off-target effects could be very different between the two.<sup>50</sup> The addition or removal of a double-stranded break in the DNA involved with the CRISPR-Cas9 system is not the only issue related to double-stranded breaks. Inaccurate repair of CRISPR-Cas9-induced DNA breaks could introduce other unintended mutations, although with the current technology this is very unlikely.<sup>51</sup>

#### Possible Risks of the CRISPR-Cas9 System: Conclusions

The possible complications of unintended base changes, mosaicism, and double-stranded breaks have the potential to create medical problems far greater than those that are needing to be fixed in the first place. For example, they could contribute to cell suicide (apoptosis) and the creation of cancer cells.<sup>52</sup>

The scientific benefits and risks of the current CRISPR-Cas9 technology are very contradictory as different trials have provided evidence for both the success and overcoming obstacles or the inability to. Each trial has its own caveat that makes the trials difficult to compare to each other. Whether or not viable or non-viable embryos were used, or how long each trial embryo was observed after and what they were testing, are all different variables that need to be taken into account when comparing the scientific benefits and risks of the CRISPR-

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<sup>50</sup> Peng, R., Lin, G., & Li, J. (2016). Potential Pitfalls of CRISPR/Cas9-mediated Genome Editing. *The FEBS Journal*, 283(7), 1218–1231. <https://doi.org/10.1111/febs.13586>.

<sup>51</sup> Kamisugi, Y., Whitaker, J. W., & Cuming, A. C. (2016). The Transcriptional Response to DNA-Double-Strand Breaks in *Physcomitrella patens*. *PLOS ONE*, 11(8), e0161204. <https://doi.org/10.1371/journal.pone.0161204>.

<sup>52</sup> Lander, E. S. (2015). Brave New Genome. *New England Journal of Medicine*, 373(1), 5–8. <https://doi.org/10.1056/NEJMp1506446>.

Cas9 system for use in humans. The most important aspects are the effects on human embryos, especially those that are viable, and whether or not any unpredicted consequences are observed.

#### Scientific Risks and Benefits: Alzheimer's Disease

In reference to Alzheimer's disease, there are risk genes and deterministic genes. The risk genes are those that increase the likelihood of inheriting the disease, and the deterministic genes directly cause the disease. There are variations in three certain genes that are inherited and will directly cause Alzheimer's disease: the amyloid precursor protein, presenilin-1 and presenilin-2. Variations in presenilin-1 are the most common cause of early-onset Alzheimer's disease. Although solely-inherited Alzheimer's disease accounts for only 1% of Alzheimer's cases, the inherited risk genes play a large role in all other cases as well. The risk gene with the largest known impact is apolipoprotein  $\epsilon$ -4 (APOE-4).<sup>53</sup> With the large genetic component of Alzheimer's, the scientific advances to the CRISPR-Cas9 genetic engineering technology could eventually lead to alleviation of Alzheimer's disease symptoms or complete eradication of the genetic mutations that cause the disease (and therefore elimination of the mutant disease genes from the germline). However, from a scientific standpoint, do the benefits outweigh the risks?

Using the data from the trials carried out with human embryos, the CRISPR-Cas9 technology is not scientifically advanced enough to use on viable human embryos before in-vitro fertilization. Although some trials have discussed and tested methods to reduce or eliminate the complications of off-target effects and mosaic mutations, they have not been 100% effective, and for use on unborn humans, there needs to be a much larger success rate than was shown (as little as one to three embryos per trial were successful without visible off-target effects). Even though genetic engineering on the human germline has progressed further than anyone thought or

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<sup>53</sup> American Alzheimer's Association. (2018). Alzheimer's Association. <https://www.alz.org/>.

imagined it would, it still is not in the stage to be used on humans. Especially with the possible off-target base changes and creation of mosaic mutations, any introduction of a mutation that would cause the embryo in question to grow up and not live a normal, healthy life (even with the original target disease edited out) may not be worth the risk (especially if we do not know what that off-target mutation may do).

### Conclusions

With more research on the technology, the reduction of these unpredicted, unwanted off-target effects, whether it be base changes, mosaic mutations, or double-stranded break errors, would be reduced. However, there does still need to be an improvement scientifically in the technology before it can really be used to alleviate or eradicate any human, genetic disease in human embryos that will later grow up and live a normal life.

Biologist Guoping Feng of MIT's McGovern Institute for Brain Research said in 2015 that he “thinks actual gene-edited humans are ‘10 to 20 years away.’”<sup>54</sup> Feng also explained that at the time, making specific edits with CRISPR, like editing or swapping DNA bases, works only about 20% of the time.<sup>55</sup> Although a couple of years have passed since this statement and the technology has improved, the percentage is still not where it needs to be for the scientific benefits of using the CRISPR-Cas9 technology on humans to outweigh the risks. One thought of scientists in the field is to combine the CRISPR-Cas9 technology with the current research in stem cells in order to generate a higher success rate. With the stem cells, scientists can edit genes

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<sup>54</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

<sup>55</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

in stem cells before turning them into an egg or sperm for fertilization.<sup>56</sup> Although this has not been done yet, it would help bring the gene editing technology a step closer for human use. With further research and improvement, however, the scientific and medical benefits could outweigh the risks of using the CRISPR-Cas9 technology on human embryos.

## **IV. Analyzing the Ethical Considerations of Genetic Engineering**

### Chapter Introduction

In this section of the thesis, I am going to analyze the ethical arguments for and against the use of the CRISPR-Cas9 technology on human embryos in order to eliminate or alleviate Alzheimer's disease. In order to accomplish this, I will present two case studies that relate to the use of the CRISPR-Cas9 engineering technology on human embryos and apply the two major theories of bioethics, deontological theory and consequentialist theory, the four major principles of medical ethics, respect for patient autonomy, beneficence, non-maleficence, and justice, and the current religious and public views to each case.

### Medical Ethics Background

The field of medical ethics applies to all clinical medicine practices and clinically-related scientific research. There are four principles to be considered in discussing the field of medical ethics: respect for patient autonomy, beneficence, non-maleficence, and justice. When considering medical ethics in terms of genetic engineering, and especially in terms of altering a human embryo, many of these theories and principles may be violated.

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<sup>56</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.



### Two Theories of Bioethics Background

It is imperative to understand the major theories of bioethics along with the four principles of medical ethics in order to analyze the ethical question at hand. There are two theories of bioethics: deontology and consequentialism. The theory of deontology was formulated by Immanuel Kant and follows rules based ethics. This theory states that we must not violate rules which depend on rational thought, and we must not intentionally harm someone else. In order to decide whether something is ethically justifiable or not, one must be capable of rational thought. In this theory, there are certain obligations and commitments that we cannot violate, regardless of the outcome (i.e. even if the outcome is ideal), and we cannot use other humans for the purpose of achieving our goals. One of the largest aspects of deontology is that we cannot lie. Kant also states, as a part of deontological theory, that no person can be simply used to fulfill one's own goals, and we cannot intentionally harm.

The consequentialist theory, formulated by Jeremy Bentham and John Stuart Mill, is quite similar to an economic theory. This theory focuses on the outcomes of behavior, and is therefore commonly contrasted to deontological theory. It judges whether something is ethically justifiable or not based on what a behavior produces and not the path it took to get to that outcome. According to consequentialism, we are trying to achieve the greatest good for the greatest number of people, which is similar to a cost-benefit analysis. Contrary to deontology, consequentialists will sacrifice individuals to save the many and achieve personal goals.

### Case Study 1: Introduction

In this section, I am answering the following question: Is it ethically justifiable to use the CRISPR-Cas9 technology, in its current condition, to eliminate risk or determinate genes of Alzheimer's disease? In order to answer this question, I propose the following scenario:

A middle-aged couple had been attempting to conceive a child and they just found out that the woman is pregnant. They have both been screened for their genetic makeup, and the father is positive for the mutated apolipoprotein  $\epsilon$ -4 (APOE-4) gene that is the major precursor for the formation of  $\beta$ -amyloid plaques, and therefore a large precursor for Alzheimer's disease. Worried about the possible inheritance of this gene, the couple decides to screen their embryo for the APOE-4 gene. They have the belief that they should do anything and everything to make sure that their child has the best and longest life possible. After screening, it is determined that the embryo also carries the mutated APOE-4 gene that is a precursor for Alzheimer's. They decide that they want to use current genetic engineering technology, the CRISPR-Cas9 system, in order to edit out the mutated gene and replace it with a normal one. However, the couple's physician is skeptical about using the technology in its current state to perform this procedure. Is it ethically justifiable for the couple and the physician to do go ahead with the editing of the embryo? My conclusion, as shown in the arguments below, is that at the current state of the CRISPR-Cas9 technology, it is not ethically justifiable for the couple to edit out the mutated APOE-4 gene from their growing embryo.

### Case Study 1: Analyzing Deontological Theory

Editing the mutated gene out of the couple's embryo would violate major aspects of deontological theory. First of all, it can be argued that the couple is not currently relying on rational thought. Although it could be argued that wanting to do everything for your child is

rational, in this case the parents are not scientists that know the small details of the unintended consequences, even if they have been informed of these consequences by their physician. They are trying to do everything possible to help their child, and even though editing out the mutated gene *is possible*, it is not rationally okay to do in its current state. As stated in the previous section, the scientific issues of unintended base changes and mosaicism could lead to consequences we have not yet discovered or predicted. Even though the couple wants to use it to possibly eliminate the risk of Alzheimer's or alleviate later symptoms of Alzheimer's in their future child, they are not considering the possible side effects. Although Alzheimer's could be gone, many other complications, which possibly could be fatal, could arise from the use of the CRISPR-Cas9 system.

From the physician and scientist standpoint, the physician would be violating deontological theory as he would be using someone else in order to reach his own goal of eliminating and/or alleviating Alzheimer's disease, especially knowing that there could be possible consequences. Deontological theory states that we cannot use others to achieve personal goals. He has the obligation to give the best possible care to each patient he encounters, and to make sure that he helps the patient become as healthy as possible. Knowing that there are possible detrimental consequences of using the CRISPR-Cas9 technology in its current state would be violating the commitment he made when he became a physician.

Kant places a lot of emphasis on the importance of the rational person, and how humans must be considered rational in order to make the decision of what is right and wrong. Around the age of 12-15, Kant allows children moral status because they are then capable of rational thought. According to this theory, embryos are not capable of rational thought and do not have moral standing. However, the embryos will eventually develop into infants and later adults with

a moral standing. Kant would originally discount the moral standing of embryos, but they do have potential to eventually develop rational thought, which must be considered in the deontological analysis of each of the following case studies. In the real world, deontological theory would not apply to these embryos that we are able to genetically manipulate until they are capable of rational thought.

Kant also states, as a part of deontological theory, that no person can be simply used to fulfill one's own goals. This applies in the sense that embryos cannot give consent at the time they would be edited, and when they reach the point of rational thought we have already violated the prohibition against using them for our own personal goals without their permission and consent.

#### Case Study 1: Analyzing Consequentialist Theory

Not only through the theory of deontology, but also through the consequentialist theory, the gene editing of this embryo would not be ethically justifiable. In this theory, we are trying to act in a way where the outcome produces the greatest good for the greatest number. In the scenario described above, the physician would be editing a gene that is in the embryo's germline, which means that the edited would be passed on to future generations. Since the CRISPR-Cas9 technology is not at the current state of a 100% success rate, or even a very high success rate, he could be causing more harm to the masses than good, which would violate this aspect of consequentialism. Through a cost-benefit analysis (as this theory is similar to economic theories), the risk of harm resulting from performing this procedure in this scenario greatly outweighs the benefits, as the unpredicted consequences could end up to be more fatal than Alzheimer's.

Unlike the theory of deontology, however, the consequentialist theory allows for sacrifice of the individual to save the many (i.e. one is able to use others to accomplish personal goals). Following this principle, it would be okay to try to edit the germline of this embryo in order to observe the outcome. Even if the outcome were detrimental rather than successful, it would still provide new research and new observations on what may happen when altering a human embryo, and therefore could help the masses in the future. However, this still would violate the other concepts of consequentialist theory.

Going along with the same concept of providing the greatest good for the greatest number, it could also be argued the opposite way, that it would not be providing good for the greatest number. Using CRISPR-Cas9, with its known complications, could easily cause more harm than good, as stated above, especially in the discussion of editing the germline and not just somatic cells. In this sense, using the technology and harming the human embryo would not provide the greatest good to the greatest number as it would not only harm the human embryo but also emotionally harm the parents and others involved in the procedure, as well as harm all of the future offspring of the embryo.

#### Case Study 1: Analyzing Non-Maleficence

The physician, in this case, is also clearly violating the medical ethics principle of non-maleficence. This principle requires that we do not intentionally harm any patient, whether it be from performing an act or not performing one at all.<sup>57</sup> We currently do not have confirmed knowledge that using the CRISPR-Cas9 system will not introduce harmful consequences, but we do know that it will introduce consequences as mosaicism and unintended base changes. With

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<sup>57</sup> McCormick, T. R. (2013). Principles of Bioethics. Retrieved from <https://depts.washington.edu/bioethx/tools/princpl.html>.

this knowledge, although we also do not know exactly what the unintended consequences may lead to in the future, the physician could not perform this gene editing procedure while also following the principle of nonmaleficence.

### Case Study 1: Analyzing Beneficence

Similar to violating the concept of producing the greatest good for the greatest number, editing out the mutant Alzheimer's gene in this embryo would violate the medical ethics principle of beneficence. Beneficence states that physicians have the duty to take steps towards removing harm from any patient and to always be of benefit to the patient.<sup>58</sup> Since CRISPR, in its current state, does not have a high success rate (i.e., a low rate of unintended consequences), the physician would not be fulfilling his duty of intentionally providing benefit and taking positive steps to help the patient, and in this case, the human embryo. On the contrary, in knowing all of the possible consequences, the physician would really be harming the patient. The principle of beneficence includes preventing harm and doing good along with removing harm. In this scenario, the physician would not be preventing harm as he, again, does not know what the possible consequences are. Although not removing the mutant gene would allow the human embryo to grow into someone who develops Alzheimer's disease, the unexpected consequences of the CRISPR-Cas9 system may cause other, more harmful consequences, and therefore performing the procedure would not be preventing harm but rather causing harm. Lastly, although it may seem as if the physician is doing good in this scenario by preventing or alleviating Alzheimer's disease in the viable human embryo, once again the unintended consequences like unexpected base changes and mosaicism may end up being worse or more

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<sup>58</sup> McCormick, T. R. (2013). Principles of Bioethics. Retrieved from <https://depts.washington.edu/bioethx/tools/princpl.html>.

quickly fatal, in which case the physician would not be doing good to the patient or the patient's family.

### Case Study 1: Analyzing Religious Views

From a religious perspective, the physician could also be construed as “playing God” in this scenario. By choosing whether or not to carry out the gene editing process, he is essentially choosing when and how the child may live and die. He is deciding whether or not the child could develop Alzheimer's disease or not, and he knows that there are possible, unpredicted detrimental outcomes if he chooses to go ahead with the procedure. In this case he is violating some essential rules of being a human being from a religious perspective because he as a person may not be in the position to decide whether to do right or wrong because he is not God.

### Case Study 1: Conclusions

In applying deontological and consequentialist theories and the principles of nonmaleficence and beneficence, and in considering some oppositional religious views, it can be concluded that with the CRISPR-Cas9 in its current technological state, it would be unethical to edit the mutant gene out of the viable human embryo in question. Even “the American Medical Association, holds that germ-line engineering shouldn't be done ‘at this time’ because it ‘affects the welfare of future generations’ and could cause ‘unpredictable and irreversible results.’”<sup>59</sup> It violates major aspects of both the deontological theory and the consequentialist theory of bioethics and therefore it cannot be concluded that it is ethically justifiable to use the CRISPR-

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<sup>59</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

Cas9 system to edit out the APOE-4 gene that is a precursor for Alzheimer's disease in the human embryo.

The main basis of concluding that it is not ethically justifiable to edit the mutant gene out of the visible human embryo in question is the fact that the CRISPR-Cas9 system is not in a scientifically current acceptable state for use on humans, and still has many problems to overcome.

### Case Study 2: Introduction

What if the CRISPR-Cas9 genetic engineering technology was acceptable for use in humans (in that the unintended consequences were completely avoided), and rarely ever made mistakes? Would it be ethically justifiable to use the CRISPR-Cas9 system, when it is at the point of an extremely low off-target effect rate, to eliminate risk or determinate genes of Alzheimer's disease? In order to answer this question, I propose the following scenario:

We project ourselves into a future in which multiple studies and trials with the CRISPR-Cas9 system have been performed, and the off-target effects like mosaicism, unpredicted base changes, etc. (the problems explained in the previous chapter) have been reduced to a 1% occurrence rate. The CRISPR-Cas9 system has recently been implemented in a small number of premier hospitals and research centers around the world, with only few in America (in large metropolitan cities as Houston, New York City, and Boston), in order to edit mutant and possibly fatal genes in genetically screened human embryos. At this point in time, the CRISPR-Cas9 system is only being used in a clinical setting to edit mutant genes that are the sources of genetic diseases that are eventually fatal or cause an extremely painful and poor quality of life. The price



to screen your fertilized embryo for the diseases that are being edited by CRISPR-Cas9, attend a diagnostic appointment with a physician and scientist who will perform the procedure, and have the procedure done with follow up appointments is extremely expensive (although the price to perform the procedure with CRISPR-Cas9 is actually very low), and only very little if any of these costs are covered by private insurance (none of the process covered by Medicare or Medicaid, or any government subsidized public insurance). “An in-vitro fertilization procedure costs about \$20,000 in the United States. Add genetic testing and egg donation or a surrogate mother, and the price soars to \$100,000,” and that is without the use of CRISPR-Cas9 to actually edit out the gene.<sup>60</sup>

A young wealthy, upper-class couple from a small town in the mid-west has one middle-school aged daughter who learned the basics of genes and DNA in her science class and asked her parents if she could be genetically screened, as one of her classmates had been and shared about it in her class. Her parents agreed and decided to also get screened. Once their results came back, both the mother and the daughter realized that they were positive for the mutated APOE-4 gene that is the major precursor for the formation of  $\beta$ -amyloid plaques, and therefore a large precursor for Alzheimer’s disease. Although the daughter did not know what that meant, the mother, who had to undergo in-vitro fertilization to have her daughter, was very worried about her daughter and her next child (as she and her husband were planning to have at least one other child).

Once the couple, who had a very good private insurance plan, was ready to have their next child, again via in-vitro fertilization, they decided to screen the fertilized embryo for the mutated APOE-4 gene. The results showed that the embryo they were planning to implant was

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<sup>60</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

positive for the mutated APOE-4 gene. The couple decided to schedule an initial appointment with the physician and scientist who could perform the CRISPR-Cas9 procedure at the nearest hospital to them, which was a two-hour flight away. The physician and scientist jointly decided that the couple was a good candidate to undergo CRISPR-Cas9 editing to edit the mutated APOE-4 gene out of their embryo. Although insurance did not cover the procedure, the couple had enough money to go forward with the process.

Even though, at this hypothetical point in time, the CRISPR-Cas9 technology is scientifically sound and makes very minimal, if any, errors, is it ethically justifiable for the couple, physician, and scientist to go through with the procedure? My conclusion, as shown in the arguments below, is that even with an almost-perfected CRISPR-Cas9 technology (almost-perfected as there are always risks of any clinical procedure), in considering the principle of justice in the distribution of scarce resources, it is still not ethically justifiable for the couple to edit out the mutated APOE-4 gene from their growing embryo until the technology is much more accessible around the world.

### Case Study 2: Analyzing Justice

Performing the clinical procedure of editing out the mutant genes with the CRISPR-Cas9 system violates the principle of justice. This principle requires the fair distribution of goods in the healthcare system, and that equal people should be able to receive equal treatment.<sup>61</sup>

According to the principle of distributive justice, the criteria for the just allocation of scarce medical resources includes the following: “to each, an equal share; to each, according to need; to

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<sup>61</sup> McCormick, T. R. (2013). Principles of Bioethics. Retrieved from <https://depts.washington.edu/bioethx/tools/princpl.html>.

each, according to effort; to each, according to contribution; to each, according to merit; and to each, according to the ability to pay.”<sup>62</sup>

In this scenario, which is a very likely case once the technology is at the point where it can successfully and accurately perform this procedure, the couple is very wealthy and has the ability to travel across the country in order to meet with the doctors that can perform the procedure, and they have the ability to pay for the whole process out of pocket (rather than it being covered by insurance). For any kind of medical procedure of the like, only the very few who can afford the travel and the cost of the medical portion itself are able to go through with this process. The accessibility is also very limited, as it is, in this scenario, only performed at very few hospitals around the world. Is this fair to those with the same problem, but not the same financial stability? “What if these improvements were only available to the richest societies, or the richest people?”<sup>63</sup> There must be some fairness in the distribution of technology and scarce resources like the CRISPR-Cas9 gene editing system.

From a financial aspect, the fairness of medical treatment is a large issue in our current society and in this specific scenario. It can be argued that it would not be ethically justifiable to perform this procedure on this specific family because this family is only one of the extreme few who could follow through with it, and then the only embryos that would have the disease edited out would be those of wealthy upper-class families. Since this clinical procedure is not covered by insurance, it would be extremely expensive. Therefore, a new gap between rich and poor would be created. In this case, Alzheimer’s disease would eventually shift to only be present in those families who were not as financially stable, as the general cost of the treatment is

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<sup>62</sup> Tong, R. (2007). *New perspectives in health care ethics: an interdisciplinary and crosscultural approach*. Upper Saddle River, N.J: Pearson/Prentice Hall.

<sup>63</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

extremely expensive. In order to remedy this issue with distributive justice, a much more expansive financial plan needs to be implemented. Whether the treatment is covered only partly or fully, it would need to be covered by both private and public insurance policies. If not covered by insurance, the price would need to be reduced or an association be created that would finance the procedure for those who do not have the funds to pay for it at the more expensive price. Without fixing the financial instability and cost, there would be no distributive justice in a financial aspect, and the gap created would render the technology ethically un-justifiable.

Another reason the procedure is only accessible in few hospitals and few countries is due to the views of genetic engineering. For example, in a study conducted in the United Kingdom, “the public’s ethical concerns [regarding genetic engineering] are associated with applications involving the use of animals or of human genetic material,” and a large portion of those concerns are based on the “unnaturalness” of altering animal and human genetic material.<sup>64</sup> In “western Europe, 15 of 22 nations prohibit the modification of the germ line. Although the United States has not officially prohibited germline modification, the US National Institutes of Health’s Recombinant DNA Advisory Committee explicitly states that it ‘will not at present entertain proposals for germ line alterations.’”<sup>65</sup> Although using the CRISPR-Cas9 technology in some of these areas may not be banned or illegal, in countries that have an extreme negative attitude towards using the technology on human genetic material, the procedure may not be accessible. Also, “in China, an invention that contravenes social moral principle cannot be granted patent

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<sup>64</sup> Frewer, L. J., Howard, C., & Shepherd, R. (1997). Public Concerns in the United Kingdom about General and Specific Applications of Genetic Engineering: Risk, Benefit, and Ethics. *Science, Technology, & Human Values*, 22(1), 98–124. <https://doi.org/10.1177/016224399702200105>.

<sup>65</sup> Lanphier, E., Urnov, F., Haecker, S. E., Werner, M., & Smolenski, J. (2015). Don’t edit the human germ line. *Nature*, 519(7544), 410–411. <https://doi.org/10.1038/519410a>.

right.”<sup>66</sup> If it is not available in certain countries or areas like this, people may have to travel further and therefore spend much more money solely on travel and lodging expenses, which would polarize the distribution of the CRISPR-Cas9 resources even more (to where it is available to a much smaller, wealthier group of people only). This would further violate the medical ethics principle of justice.

From a healthcare perspective, the distribution of this resource would also be extremely unfair and unequal. This procedure would have to be in effect with little risk for a long period of time before it would be largely or fully covered by private healthcare companies, and even much longer for public or government subsidized healthcare policies. Therefore, even if it were partially covered by healthcare, only those with very good, private healthcare plans would be able to afford the procedure. Therefore, only the wealthy who are able to pay for the more expensive and higher-coverage healthcare plans would be truly able to pay, so the only people able that would have the procedure covered by their healthcare plan would be those who could probably afford to pay for it out-of-pocket in the first place. It would only further the two-class system of healthcare. The coverage of this procedure by all healthcare plans is very, very far into the future (if it would even happen at all), especially with how new the technology is, even after more years of research to when the technology makes very little to no off-target, unpredicted changes. There would be no distributive justice under these circumstances.

### Case Study 2: Analyzing Deontological Theory

From a deontological perspective, editing the mutated APOE-4 gene out of the couple’s new, in-vitro, viable embryo would still not be ethically justifiable. One main statement of

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<sup>66</sup> Peng, Y. (2016). The morality and ethics governing CRISPR–Cas9 patents in China. *Nature Biotechnology*, 34(6), 616–618. <https://doi.org/10.1038/nbt.3590>.

deontological theory is that we must not violate rules, and there are certain obligations and commitments we cannot violate, regardless of outcome. Two of the rules is that we may not use others to achieve personal goals, and that one must be of moral standing and have rational thought to make decisions (especially those of right or wrong). In this case, the argument is the same as in the first case study. The embryo is not able to make its own decisions, as it does not yet have moral standing and cannot make rational thoughts. Although it will develop into a human who can, at the moment it cannot, so the parents would be making their decision based on what they think is best. This would be fulfilling their goals through using the child.

The CRISPR-Cas9 system “is likely to power a new generation of gene treatments for serious diseases,” but is that it?<sup>67</sup> One of the main concerns of the public (whether CRISPR-Cas9 is scientifically accurate or not) is that the CRISPR-Cas9 technology will be used for editing that goes much further than editing out mutant genes that cause terrible diseases, for example, using it to create “a dystopia of super-people and designer babies for those who can afford it,” and down a further slippery slope in which “most of the public does not appreciate.”<sup>68</sup> The main fear is that “if germ-line engineering becomes part of medical practice, it could lead to transformative changes in human well-being, with consequences to people’s life-span, identity, and economic output.”<sup>69</sup> The negative views of CRISPR-Cas9 seem to be becoming even more negative as the thoughts of “designer babies” and “editing the human race” have been coming

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<sup>67</sup> Regalado, A. (2016). Top U.S. Intelligence Official Calls Gene Editing a WMD Threat. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/600774/top-us-intelligence-official-calls-gene-editing-a-wmd-threat/>.

<sup>68</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

<sup>69</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

into light.

Although the CRISPR-Cas9 system is being analyzed in order to eliminate or alleviate disease, specifically Alzheimer's disease, would even just that lead to an era of human augmentation and enhancement? For example, people with a specific variant of the Amyloid precursor protein are protected against Alzheimer's and dementia, so they remain very sharp in old age compared to those who do not have the variant. Using CRISPR-Cas9 to introduce that variant into someone could be construed as enhancing that human rather than just protecting him or her from a harmful disease.<sup>70</sup> The use of this technology to make people just a bit sharper, smarter, more athletic, more disease-resistant, etc. would be bordering, if not crossing the line of what is considered an acceptable use of this technology. However, humans with rational thought did create this technology, so its use, in any scenario, could be argued to be rational, even though that argument does not outweigh the argument against using CRISPR-Cas9 for anything but disease prevention.

### Case Study 2: Analyzing Consequentialist Theory

Consequentialist theory can be used to argue both for and against the ethical justifiability of use of CRISPER-Cas9. From one standpoint of a consequentialist theorist, using the CRISPR-Cas9 technology to edit out the mutant APOE-4 gene in this scenario (where the technology is scientifically sound and extremely accurate), is not ethically justifiable. The consequentialist theory requires that we act in a way where the outcome produces the greatest good for the

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<sup>70</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

greatest number of people. Using this technology would create the greatest good for the greatest number by editing out the mutant APOE-4 gene, but if the technology went any further than this it could have the ability to eliminate a certain population of certain gene pool which would do the opposite of what consequentialist theory calls to do. Eliminating a certain gene pool or certain physical aspects would lead to less diversity and variety around the world, which would not be providing the greatest good for the greatest number.

From another standpoint of a consequentialist theorist, using the CRISPR-Cas9 technology to edit out the mutant APOE-4 gene in this scenario is ethically justifiable. As stated above, the consequentialist theory calls to act in a way where the outcome produces the greatest good for the greatest number of people, regardless of the steps it took to get to the outcome. In any case where the germ-line, and therefore what will be passed on to later generations, is edited, any gene editing technology must be questioned.

However, in this scenario, eliminating or even just alleviating Alzheimer's disease in this one embryo would produce greater good than harm for the greatest number of people, as the parents are happy and the viable embryo will have a less extreme case of Alzheimer's disease or not develop Alzheimer's disease at all (and will also affect later generations). Also, through the argument of possibly making enhanced humans, whether it be with greater intelligence or a different trait (even though it is still not completely clear how genetics affect individual intelligence), this technology could cause further developments and innovations that could change the world for the better by increasing problem-solving and entrepreneurial skills.<sup>71</sup> Although not the same as in the last chapter, this could still bring about very different unintended

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<sup>71</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.



consequences for the human race, as certain children would be more intelligent or superior in some other way due to the ability of the parents to afford the procedure. But this begs the question, are we in a post-ethics technological world? Through analyzing the current views and arguments on the CRISPR-Cas9 technology, I personally do not yet think we are because the topic of editing humans, and many other topics similar, are still highly debated and thought of negatively. However, we could be on the path towards one.

### Case Study 2: Analyzing Religion

From a religious standpoint, performing this procedure may be construed as human beings “playing God,” and therefore violating the rules of human life. Certain areas of the world have different religious-based opinions on this technology. For example, “life science research in China does not encounter the same religious objections compared with the West and research based on CRISPR–Cas9 technology, such as using such gene-editing technology in human 3PN zygotes, does not provoke the same level of controversy.”<sup>72</sup> The Christian Research Institute, in contrast, states that

“Christians must focus on questions of disease versus enhancement, the purpose(s) of medicine, and the dangers involved in possibly releasing new (and unintended) harmful genes into the human gene pool. The primary purpose of

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<sup>72</sup> Peng, Y. (2016). The morality and ethics governing CRISPR–Cas9 patents in China. *Nature Biotechnology*, 34(6), 616–618. <https://doi.org/10.1038/nbt.3590>.

medicine ought to be, in Nigel Cameron's words, 'a tradition of healing.'"<sup>73</sup>

The Christian Research Institute also explains that through the analysis of Christian Doctrine, "We lack both the wisdom and purity necessary to decide matters of human 'perfection.' It is, therefore, immoral to use such genetic technologies as human eugenics."<sup>74</sup> These statements and arguments are discussed due to the ability to pick and choose genes to be edited before a human is even born, which is also one of the many reasons why gene editing was added to the threat list as a possible "weapon of mass destruction and proliferation" by the US intelligence community in 2016.<sup>75</sup>

### Case Study 2: Conclusions

Using the CRISPR-Cas9 engineering technology to solely edit out detrimental, mutant disease genes, like the mutant genes that cause Alzheimer's disease, is ethically justifiable when the technology is in a state of almost perfect accuracy. However, there are still major problems relating to justice, as well as violations of deontological and consequentialist theory in the analysis of this case study. Not only this, but there are also large fears of other use of the technology (creating a slippery slope) that would not render it ethically justifiable even if it

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<sup>73</sup> McKenzie, M. (2009, April 21). The Christian and Genetic Engineering. Retrieved from <http://www.equip.org/article/the-christian-and-genetic-engineering/>.

<sup>74</sup> McKenzie, M. (2009, April 21). The Christian and Genetic Engineering. Retrieved from <http://www.equip.org/article/the-christian-and-genetic-engineering/>.

<sup>75</sup> Regalado, A. (2016). Top U.S. Intelligence Official Calls Gene Editing a WMD Threat. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/600774/top-us-intelligence-official-calls-gene-editing-a-wmd-threat/>.

is in a state of perfect accuracy, as it could also affect the human race and evolution through other, more physically apparent edits.

#### Chapter 4 Conclusions

From the two case studies discussed above, it can be concluded that it is not ethically justifiable to use CRISPR to edit the germline of a human embryo. The violations of the most of the principles of medical ethics (the first case study relates to beneficence and nonmaleficence, and the second relates to justice), as well as of deontological and consequentialist theories, greatly outweigh the ethical benefits of using the technology on human embryos, whether it be in the technology's current state or in a state where detrimental side effects have been eliminated and it is at an almost-perfect accuracy.

Although I concluded that the CRISPR-Cas9 gene editing technology is not ethically justifiable for use in either scenario, a hard set of limits of where application of this technology is ethically justifiable could be introduced that would make the public have a more positive view of not only the CRISPR-Cas9 technology but also any other genetic engineering technology for use on humans.

## V. Proposed Limitations of the CRISPR-Cas9 System

*“Scientists are developing ways to edit the DNA of tomorrow’s children. Should they stop before it’s too late?”<sup>76</sup>*

The limits that need to be set on the CRISPR-Cas9 technology, when used to modify the germline of human subjects, range across all aspects of the ethical arguments outlined above. Although we currently are not in an age where this technology is openly and widely used on humans, the limitations for future use need to be addressed as the technology is coming closer and closer to human use every day. The main limitation, and the most concerning to the general public, would address how far we should be allowed to take this technology and what exactly we should use the technology for.

The use of genetic engineering, and especially the CRISPR-Cas9 system, on humans could be used for much more than editing out and replacing one specific gene. If we were to develop the technology to an almost perfect accuracy, using it to edit out and replace genes that would cause detrimental and possibly fatal diseases would be the best possible outcome. However, once the technology gets that far it could and may also be used to many other

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<sup>76</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

procedures. For example, if we were able to edit out a gene causing Alzheimer's disease and replace it with a non-mutated gene, we could also edit out a gene for a certain physical trait and replace it with a different physical trait. "There are moral and ethical issues, but one of the profound questions is just the appreciation that if germ-line editing is conducted in humans, that is changing human evolution."<sup>77</sup> Although most physical traits like hair color, skin color, eye color, etc. are determined by the combination of multiple genes, even changing one of those genes could very likely lead to the embryo being born with the desired trait or a trait much more similar to the desired trait than the original. The genetic determination of other human traits, like athleticism and intelligence, is not fully known for humans, although it is known that genetics play some role for both of them and other traits of the like. There is constant research on these traits and their genetic components, so with more research in the time it would take to perfect the CRISPR-Cas9 technology, editing genes in human embryos to make the child smarter or more intelligent could be a realistic possibility. These thoughts culminate into the main fear of the public: using this technology to have superior children, or "designer babies."<sup>78</sup>

Playing into this fear would also be the financial aspect of using genetic engineering to alter the genetic makeup of unborn children. Using the technology on humans would be very expensive, and as described above not fully covered by insurance, so only the wealthiest would be able to use it. Therefore, the wealthiest people would be able to create superior children, which would not only further the gap between rich and poor, but also cause a more controversial class divide. It could also eliminate certain physical traits and certain aspects of the human race around the world to create children with the "desired" traits. There would be no distributive

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<sup>77</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

<sup>78</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

justice in this case. This is why it is imperative to set limits on this technology, the first being what it is used for. Genetic engineering technology like CRISPR-Cas9 and the like should be limited to use for disease control and elimination. It should not be allowed to use this technology for human enhancement (physical looks, intelligence, athleticism, etc.). This limitation essentially removes the main public fear of the use of any genetic engineering technology on human embryos. However, as humans created this technology and are furthering its development and use, it may be unrealistic to assume that these limitations would be set.

Along with this limitation comes the financial limitations. Although many aspects of the healthcare system are not equally fair among everyone who needs them, using the CRISPR-Cas9 technology to prevent disease should be at least partially covered by private and public healthcare plans so that any member of the general public has the chance to save their future child from a fatal disease. This would allow justice within the healthcare system (in relation to genetic engineering), the greatest good for the greatest number of people, and a higher rate of disease elimination, which is the ultimate goal. Although these limitations and regulations of the technology could ethically justify its use on human embryos, and “that in the United States, there are [currently] piles of regulations to keep lab science from morphing into a genetically modified baby anytime soon,” the future cannot be accurately predicted and it is unknown whether limitations would be set once the technology reaches the necessary point for human use.<sup>79</sup>

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<sup>79</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

## VI. Conclusion

In this thesis I attempted to explore the CRISPR-Cas9 genetic engineering system to modify the germline of human embryos in order to eliminate or alleviate Alzheimer's disease. Through this exploration, I analyzed the specifics of the technology in its current state, the causes of Alzheimer's disease, the scientific benefits and risks, and the ethical arguments for and against its use on humans. Through the explanations and arguments discussed in the previous section, I conclude that overall, it is not ethically justifiable to use the CRISPR-Cas9 technology to edit the germline, and therefore the hereditary material to be passed on to all future offspring, of a human embryo.

As previously explained, the chapter on scientific benefits and risks, although the CRISPR-Cas9 system is the most developed engineering technology (and that which is closest to use on humans), it is not nearly perfected to the necessary point of accuracy in which it would not cause more harm than good. The possible side effects of complications like unintended base changes or mosaicism are unknown, and could possibly cause harm that far outweighs the benefit, for example, how it can cause cancer.<sup>80</sup> The science behind the CRISPR-Cas9 technology is still in a developmental stage when considering its use for human embryos. This conclusion is based on the analysis of multiple trials and case studies that have been performed by scientists and experts on genetic engineering across the world, none of which have been able to successfully edit out a gene from an inviable human embryo without any side effects in the

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<sup>80</sup> Lander, E. S. (2015). Brave New Genome. *New England Journal of Medicine*, 373(1), 5–8. <https://doi.org/10.1056/NEJMp1506446>.

future (as none have observed the long-term effects or tried to use the CRISPR-Cas9 system on viable human embryos).

Not only through the scientific risks, but the ethical arguments against the technology for use on humans also far outweighs the ethical arguments for the use of genetic engineering technology on human embryos. The technology violates the bioethical theories of deontology and consequentialism, as well as some of the four principles of medical ethics: justice, beneficence, and non-maleficence. The financial and low availability aspects of the clinical use of the technology do not allow for distributive justice, and create unfair gaps between those who can afford all expenses and those who cannot. As stated previously, the CRISPR-Cas9 genetic engineering system is considered to be “a slippery slope toward much more unacceptable uses.”<sup>81</sup> There is a large fear of the creation of “designer babies,” which are superior children who have physical and possibly mental traits that were chosen before birth.<sup>82</sup> This also creates the fear of a superior race, an elimination of certain traits of humans, and a larger gap between rich and poor. Using the CRISPR-Cas9 genetic engineering system on humans also violates different religious doctrines, as explained through the example of the Christian doctrine in chapter four.

Even if the technology were developed to an almost perfect accuracy to where the scientific benefits outweigh the risks, the ethical arguments still would form the conclusion that overall, it is not ethically justifiable to use the CRISPR-Cas9 technology to edit the genetic makeup of a human embryo affecting its germline. The necessary limitations outlined in chapter four would need to be put in place in order to ethically justify CRISPR-Cas9 use on humans, and

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<sup>81</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

<sup>82</sup> Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.



as the future cannot be predicted, at this time it is still not ethically justifiable to use the CRISPR-Cas9 technology to edit the genetic makeup of a human embryo. However, once the CRISPR-Cas9 genetic engineering system reaches the scientific point for use on humans, we may see necessary limitations implemented and therefore the main fears of using genetic engineering on humans would be alleviated. It is not unlikely that it will be clinically used in the future for the benefit of the human race through the alleviation and possible elimination of Alzheimer's disease, along with other genetically caused diseases and medical complications.

## **Bibliography**

## Articles

Baltimore, D., Berg, P., Botchan, M., Carroll, D., Charo, R. A., Church, G., ... Yamamoto, K. R.

(2015). A prudent path forward for genomic engineering and germline gene modification.

*Science*, 348(6230), 36–38. <https://doi.org/10.1126/science.aab1028>.

*This article discusses the background of genetic engineering and CRIPSR, as well as current applications for the technology. It also contains a section regarding moving forward and mentions changing a disease-causing genetic mutation. It concludes that the public must trust science in general in order for this technology to move forward in its application.*

Lander, E. S. (2015). Brave New Genome. *New England Journal of Medicine*, 373(1), 5–8.

<https://doi.org/10.1056/NEJMp1506446>.

*This article gives the background and small history of the recombinant-DNA revolution and discoveries of how certain bacteria can be incorporated into the Cas9 system. It also presents some challenges to the technology, including unwanted edits in a different part of the genome, and discusses editing certain somatic genes vs. the genome that will be passed down to offspring. It also incorporates Alzheimer's with the DNA editing technology.*

Tu, Z., Yang, W., Yan, S., Guo, X., & Li, X.-J. (2015). CRISPR/Cas9: a powerful genetic engineering

tool for establishing large animal models of neurodegenerative diseases. *Molecular*

*Neurodegeneration*, 10(1). <https://doi.org/10.1186/s13024-015-0031-x>.

*This article discusses current large animal models of neurodegenerative diseases and how CRISPR-Cas9 can be used as a tool in these models. According to this research, CRISPR-Cas9 has already been applied to multiple animals for RNA editing. It discusses advantages and limitations to the CRISPR-Cas9 system. They concluded that CRISPR-Cas9 can successfully be used to generate genetic mutations in large animals that mimic humans.*

Tu, Z., Yang, W., Yan, S., Yin, A., Gao, J., Liu, X., ... Li, X.-J. (2017). Promoting Cas9 degradation reduces mosaic mutations in non-human primate embryos. *Scientific Reports*, 7, 42081.

<https://doi.org/10.1038/srep42081>.

*This article describes the CRISPR-Cas9 system and its contributions to mosaicism in non-human embryos. It discusses the possibility of degrading the remaining Cas9 protein that could be causing the mosaicism by tagging it with ubiquitin. They found that shortening the half-life of the Cas9 protein reduces mosaicism in non-human embryos.*

Liang, P., Xu, Y., Zhang, X., Ding, C., Huang, R., Zhang, Z., ... Huang, J. (2015). CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. *Protein & Cell*, 6(5), 363–372.

<https://doi.org/10.1007/s13238-015-0153-5>.

*This article discusses using CRISPR-Cas9 to modify animal zygotes and human cells, but there is still research to be done in order to successfully apply this model to humans for clinical use where it would not cause unwanted, off-target effects. There is an in-depth discussion of CRISPR-Cas9 editing of the HBB gene in human tripronuclear zygotes.*

Lanphier, E., Urnov, F., Haecker, S. E., Werner, M., & Smolenski, J. (2015). Don't edit the human germ line. *Nature*, 519(7544), 410–411. <https://doi.org/10.1038/519410a>.

*This article starts off stating that there are grave ethical concerns regarding this research. It discusses the possible application in HIV, as well as the distinction between editing the genome vs. just certain somatic genes. One social concern that it touches on is the accessibility and acceptance in certain societies compared to others.*

Reardon, S. (2016). First CRISPR clinical trial gets green light from US panel. *Nature*.

<https://doi.org/10.1038/nature.2016.20137>.

*This article discusses the first CRISPR clinical trial in the US that will be funded by a \$250 million immunotherapy foundation fund. This clinical trial is on humans, and will remove T cells from humans with cancers and will remove a gene for a protein that identifies T cells as human immune cells and prevent the cancer from disabling them.*

Nowakowski, A., Walczak, P., Janowski, M., & Lukomska, B. (2015). Genetic Engineering of Mesenchymal Stem Cells for Regenerative Medicine. *Stem Cells and Development*, 24(19), 2219–2242. <https://doi.org/10.1089/scd.2015.0062>.

*This article discusses the clinical application of genetically engineering Mesenchymal stem cells, including how it will affect cell lineages and the financial aspect of the medical application. It also discusses genetic engineering in other types of stem cells and their clinical application. The safety aspect of this treatment is something the authors want to research further and believe it will be of great concern in future application.*

Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, 346(6213), 1258096–1258096. <https://doi.org/10.1126/science.1258096>.

*Jennifer Duodna is one of the leaders in determining the function, uses, and pathways of the CRISPR-Cas9 system. She dives deeply into the background of the CRISPR-Cas9 system, and even begins to discuss CRISPRi (CRISPR interference), which can block transcriptional elongation. She proposes that this technique will be able to be used in mammalian cells.*

Frewer, L. J., Howard, C., & Shepherd, R. (1997). Public Concerns in the United Kingdom about General and Specific Applications of Genetic Engineering: Risk, Benefit, and Ethics. *Science, Technology, & Human Values*, 22(1), 98–124. <https://doi.org/10.1177/016224399702200105>.

*This article was published in order to publicize the concerns of the United Kingdom public regarding the application and use of genetic engineering. Most of the public was determined to be concerned with human application of genetic engineering, and the public attitude of genetic engineering is vital in order to advance the technology any further. People were concerned about genetic engineering for multiple different reasons.*

Macnaghten, P. (2004). Animals in their Nature: A Case Study on Public Attitudes to Animals, Genetic Modification and ‘Nature.’ *Sociology*, 38(3), 533–551. <https://doi.org/10.1177/0038038504043217>.

*This article discusses the ethical and social controversies of genetically modified animals and how other people feel towards them. The article concludes that there is and will be controversy and not immediate acceptance of genetically modified animals, mainly due to a “non-natural” interference with nature and animal testing.*

Peng, Y. (2016). The morality and ethics governing CRISPR–Cas9 patents in China. *Nature Biotechnology*, 34(6), 616–618. <https://doi.org/10.1038/nbt.3590>.

*This article discusses patent law in China and how there may be complications with it in the field of genetic engineering due to the ethical concerns that CRISPR-Cas9 raises for humans. It mentions the creation of designer plants and animals (“designer pets”) and their difference from the United States in the patent process for a technology that has so many moral and ethical concerns.*

Iqbal, K., Liu, F., Gong, C.-X., & Grundke-Iqbal, I. (2010). Tau in Alzheimer disease and related tauopathies. *Current Alzheimer Research*, 7(8), 656–664.

*This source discusses the hyperphosphorylated tau protein and its contribution to progressive Alzheimer’s disease. It discusses, in detail, the MAP and MAPT genes that are involved with the hyperphosphorylated tau proteins, and compares brain structure of normal tau brains to hyperphosphorylated tau brains.*

Gong, C.-X., & Iqbal, K. (2008). Hyperphosphorylation of Microtubule-Associated Protein Tau: A Promising Therapeutic Target for Alzheimer Disease. *Current Medicinal Chemistry*, 15(23), 2321–2328. <https://doi.org/10.2174/092986708785909111>.

*This source also discusses the hyperphosphorylated tau protein in relation to progressive Alzheimer’s disease. It discusses the symptoms of Alzheimer’s and the different possible genetic and pharmaceutical therapies through the alteration of naturally occurring in the body.*

Shoghi-Jadid, K., Small, G. W., Agdeppa, E. D., Kepe, V., Ercoli, L. M., Siddarth, P., ... Barrio, J. R. (2002). Localization of Neurofibrillary Tangles and Beta-Amyloid Plaques in the Brains of Living Patients with Alzheimer Disease. *The American Journal of Geriatric Psychiatry*, 10(1), 24–35. <https://doi.org/10.1097/00019442-200201000-00004>.

*This article quickly introduces the symptoms and simple two causes of Alzheimer’s disease. It lists the genetic factors and proteins that are involved with Alzheimer’s disease, and discusses an experiment report regarding neurofibrillary tangles and  $\beta$ -amyloid plaques in living Alzheimer’s patients.*

Park, A. (2017, August 7). U.S. Scientists Use CRISPR to Fix Genetic Disease in Human Embryos for the First Time. *Time Magazine*. Retrieved from <http://time.com/4882855/crispr-gene-editing-human-embryo/>.

*This article discusses the first human embryo application of the CRISPR-Cas9 system in order to fix a genetic disease. This application was performed by Chinese scientists and proved that using CRISPR-Cas9 for this purpose is possible, however, it was not 100% successful as some of the embryos were not viable.*

Liu, C.-C., Liu, C.-C., Kanekiyo, T., Xu, H., & Bu, G. (2013). Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nature Reviews. Neurology*, 9(2), 106–118. <https://doi.org/10.1038/nrneurol.2012.263>.

*This source discusses the APOE protein in regards to Alzheimer's risk. It discusses the different types of APOE, as well as its genetic contribution to the development of  $\beta$ -amyloid plaques. It also talks about the possible therapy techniques regarding APOE.*

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group\* under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*, 34(7), 939–939. <https://doi.org/10.1212/WNL.34.7.939>.

*This article, written by all M.D.'s, discusses the characterizations of Alzheimer's disease and gives the clinical criteria for Alzheimer's disease diagnosis. It also discusses criteria for other forms of dementia and the necessity of taking medical history from a patient when diagnosing Alzheimer's and related diseases.*

Regalado, A. (2015). Engineering the Perfect Baby. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/>.

*This article discusses the progress of gene editing, specifically through the editing of human embryos. Regalado addresses different leaders in the field who he has spoken to, and he also brings up the ethical component of using CRISPR-Cas9 as a treatment (and exactly what it should be used for).*

Regalado, A. (2016). Top U.S. Intelligence Official Calls Gene Editing a WMD Threat. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/600774/top-us-intelligence-official-calls-gene-editing-a-wmd-threat/>.

*This article discusses the threats of genetic engineering and how the United States has addressed them. It explains how our current genetic engineering technologies have been deemed weapons of mass destruction by the United States due to the possible outcomes of their use.*

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., ... Cole, G. (1996).

Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science (New York, N.Y.)*, 274(5284), 99–102.

*This article discusses the  $\beta$ -amyloid plaques and how they are formed. It discusses the memory deficits due to  $\beta$ -amyloid plaques in transgenic mice, and goes into great scientific detail about the  $\beta$ -amyloid plaques.*

Connor, S. (2017). First Human Embryos Edited in U.S. *MIT Technology Review*. Retrieved from <https://www.technologyreview.com/s/608350/first-human-embryos-edited-in-us/>.

*This article discusses the trial that conducted the first editing of a human embryo in the United States with the CRISPR-Cas9 system. It discusses the outcome of the trial as well as possible fears and ideas the success of the trial created.*

Peng, R., Lin, G., & Li, J. (2016). Potential pitfalls of CRISPR/Cas9-mediated genome editing. *The FEBS Journal*, 283(7), 1218–1231. <https://doi.org/10.1111/febs.13586>.

*This article discusses the rapid development of the CRISPR-Cas9 system and the many possible consequences of its use. It discusses different strategies for possibly confronting the problems and off-target effects caused by CRISPR-Cas9.*

Kamisugi, Y., Whitaker, J. W., & Cuming, A. C. (2016). The Transcriptional Response to DNA-Double-Strand Breaks in *Physcomitrella patens*. *PLOS ONE*, *11*(8), e0161204.

<https://doi.org/10.1371/journal.pone.0161204>.

*This article was used in order to discuss double-stranded breaks, and how to repair. The article discusses DNA double-stranded breaks in the bryophyte Physcomitrella patens and the pathways this type of plant uses to repair double-stranded breaks.*

#### Other Books:

Goodfield, J. (1979). *Playing God: genetic engineering and the manipulation of life*. New York:

Harper & Row.

Harris, J. (1998). *Clones, genes, and immortality: ethics and the genetic revolution*. Oxford ; New

York: Oxford University Press.

Harris, J. (1992). *Wonderwoman and Superman: the ethics of human biotechnology*. Oxford

[England] ; New York: Oxford University Press.

Reiss, M. J., & Straughan, R. (1996). *Improving nature? the science and ethics of genetic*

*engineering*. Cambridge [England]; New York, NY, USA: Cambridge University Press.

Tong, R. (2007). *New perspectives in health care ethics: an interdisciplinary and crosscultural*

*approach*. Upper Saddle River, N.J: Pearson/Prentice Hall.

#### Websites

CRISPR/Cas9 guide. AddGene. <https://www.addgene.org/crispr/guide/>.

Mayo Clinic Staff. (2017, August 11). Alzheimer's Disease: Symptoms and Cause. Retrieved from

[https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/symptoms-causes/syc-](https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/symptoms-causes/syc-20350447)

[20350447](https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/symptoms-causes/syc-20350447).



- Dacks, P., PhD. (2016, November 16). What APOE Means for Your Health. Retrieved from <https://www.alzdiscovery.org/cognitive-vitality/blog/what-apoe-means-for-your-health>.
- Brown, H. (2015, April 26). World's First Genetically Modified Human Embryo Raises Ethical Concerns. <http://theconversation.com/worlds-first-genetically-modified-human-embryo-raises-ethical-concerns-40766>.
- McCormick, T. R. (2013). Principles of Bioethics. Retrieved from <https://depts.washington.edu/bioethx/tools/princpl.html>.
- Meštrović, T., M.D.,PhD. (2016, January 13). How Does CRISPR Compare to Other Gene-Editing Techniques? Retrieved from <https://www.news-medical.net/life-sciences/How-Does-CRISPR-Compare-to-Other-Gene-Editing-Techniques.aspx>.
- Belluz, J., & Irfan, U. (2017, October 25). Two new CRISPR tools overcome the scariest parts of gene editing. Retrieved from <https://www.vox.com/2017/10/25/16527370/crispr-gene-editing-harvard-mit-broad>.
- Le Page, M. (2017, March 9). First Results of CRISPR Gene Editing of Normal Embryos Released. Retrieved from <https://www.newscientist.com/article/2123973-first-results-of-crispr-gene-editing-of-normal-embryos-released/>.
- Le Page, M. (2017, March 15). Mosaic Problem Stands in the Way of Gene Editing Embryos. Retrieved from <https://www.newscientist.com/article/mg23331174-400-mosaic-problem-stands-in-the-way-of-gene-editing-embryos/>.
- The Jackson Laboratory. (2018). Mosaicism. Retrieved from <https://www.jax.org/jax-mice-and-services/model-generation-services/crispr-cas9/mosaicism#>.
- McKenzie, M. (2009, April 21). The Christian and Genetic Engineering. Retrieved from <http://www.equip.org/article/the-christian-and-genetic-engineering/>.

Memorial Şişli IVF & Genetics Center. Preimplantation Genetic Diagnosis by Next Generation Sequencing (NGS). Retrieved from <http://www.tupbebek-genetik.com/en/genetics/next-generation-sequencing-ngs/>.

OriGene Technologies, Inc. (2018). CRISPR-Cas9, Gene Editing Tool. Retrieved from [www.origene.com/products/gene-expression/crispr-cas9](http://www.origene.com/products/gene-expression/crispr-cas9).

Other Helpful Organizations:

American Alzheimer's Association  
Alzheimer's Drug Discovery Association  
Christian Doctrine  
Various Case Studies  
Chat Forums and Online Help/Discussion Groups