THE ETHICS BEHIND UTILIZATION OF GERMLINE GENE EDITING IN MEDICINE

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Abstract

Genetic editing has arguably been one of the most significant breakthroughs in the scientific world in recent years. Its utilizations are practically infinite, ranging from preventing genetic disorders, engineering drought-resistant crops, and finding cures for diseases like cancer. The discovery of CRISPR, or clustered regularly interspaced short palindromic repeats, was a breakthrough in itself. CRISPR-Cas9, a variant of CRISPR, has been used successfully for several gene therapy clinical trials. These trials used somatic genetic editing, which are mutations of the genome that cannot be passed on to future generations. The success of these clinical trials opened the door to the possibility of creating more targeted, permanent genetic changes. However, introducing mutations into the genome that can be passed down to future generations, known as germline genetic editing, has brought forth an ethical debate. The majority of the scientific community believes the ethical implications of germline gene editing must be resolved before it can be legalized for widespread use. Regarding the ethics of utilizing germline gene editing in the medical field, more research, including successful clinical trials, must be performed before the practice becomes legal for widespread patient use.

Introduction

Genetic editing was first utilized in 1962 by William Szybalski when he inserted foreign DNA into mammalian cells to fix a genetic mutation (Szybalska, 1962). Traditional gene therapy utilizes viral vectors to induce mutations in the genome of human subjects (Ibraheem, 2014). However, several safety concerns and fatalities using viral methods caused a halt in the pursuit of using gene therapy in medicine until the discovery of CRISPR (Stolberg, 1999). Nicknamed "molecular scissors" by scientists, CRISPR's original role was to protect bacteria against invasion by foreign DNA. Scientists quickly realized that if they could program these "scissors" to induce double-stranded breaks in DNA at precise locations and create the correct sequence of RNA, they would be able to alter their choice of areas in the genome of organisms. However, this first variant of CRISPR that was discovered was far too primitive and unreliable to be used in human subjects, eventually leading to the creation of CRISPR-Cas9. This breakthrough in gene editing technology, which now has the power to be used to change the genome in a way that can be inherited, comes with a plethora of ethical concerns. The goal of this review is to (1) delve into the history and mechanism of CRISPR-Cas9, (2) discuss the ethical considerations around using CRISPR-Cas9 to make heritable edits to the genome for medical advancement, (3) showcase a clinical trial involving germline gene therapy, and (4) discuss the future of utilizing CRISPR-Cas9 in medicine.

A History of CRISPR-Cas9

CRISPR was first discovered in 1987, but the name CRISPR was not coined until 2002 (Jansen, 2002). Because of CRISPR's lack of precision, scientists looked for different variants of CRISPR to see if they could develop one that was successful enough to be used for gene editing in human

subjects. In 2005, CRISPR-Cas9 was discovered in prokaryotic cells, with an immune role in the cell (Mojica, 2005). A few short years later, CRISPR-Cas9 was used to edit mammalian cells successfully (Mali, 2013). CRISPR-Cas9 was revolutionary for three reasons: it could be used in situ, it was easy to use, and it was relatively accurate. Several labs and scientists saw the lucrative potential of CRISPR-Cas9 and rushed to be the first to obtain a patent for it. More than one scientist filed for a patent simultaneously, the first being Jennifer Doudna from the University of California at Berkeley. Unfortunately for Doudna, the first patent was granted to Feng Zhang of the Broad Institute, despite the fact that Doudna had filed for her patent nearly seven months before Zhang. Eventually, after disagreement from both parties and legal proceedings, a patent was also granted to Doudna. In the United States, several companies were licensed through Zhang, and several were licensed through Doudna. In 2022, the U. S. Patent and Trademark Office Appeal Board changed its mind regarding Doudna's patent for the usage of CRISPR-Cas9 in human cells, leading to financial challenges for any companies licensed through Doudna, plus an even bigger legal headache for Doudna and her team. It was a different story for Doudna overseas, as she won a similar battle and holds patents for the CRISPR-Cas9 system in the United Kingdom, Japan, Australia, China, New Zealand, and Mexico. Another key event in CRISPR-Cas9's short history occurred in 2020- Jennifer Doudna and Emmanuelle Charpentier made history as the first women to be solely awarded the Nobel Prize in Chemistry for their work with CRISPR-Cas9.

This technology is seen by most as something that can be utilized to permanently alter the human genome in the future, but not in the present. Several years ago in China, a controversial experiment regarding human immunodeficiency virus, or HIV, resistance took place. In

November 2018, a scientist named Jiankui He published a study detailing his use of CRISPR-Cas9 to edit the germline of embryos to produce HIV-resistant twins (Kolata, 2018). This sent the scientific community into an uproar, as this study crossed several ethical boundaries and used CRISPR-Cas9 in a risky, uncharted way. Almost everything about the experiment was unethical, even how the volunteers were recruited. He posted an ad on a popular social media platform to find couples who were interested in participating in his study to create babies who were unable to contract HIV. The qualifications were simply to be a heterosexual married couple with an HIV-positive husband and an HIV-negative wife and for both husband and wife to have university-level degrees. He wished for the risk of HIV contraction during IVF conception to be minimal, so he recruited these couples in hopes they could be persuaded to participate for fear of contracting HIV during sexual intercourse. Despite there being known, safe, ethical methods for preventing HIV transmission to babies, He still conducted this experiment. These babies are the first of their kind to be born, and significant data has been released about them. The twins are referred to as Nala and Lulu, as not to disclose their real names. It is known that Nala has both copies of the *CCR5* gene inactivated. Both copies of the *CCR5* gene must be inactivated to prevent HIV since, with no active copies, the CCR5 receptors that the HIV virus binds to will not be synthesized. Unfortunately, Lulu only has one inactivated copy of the *CCR5* gene, so she will not be protected against HIV like her sister Nala. In addition to the twins, another baby was born, and even less is known about this third baby; most likely, this is due to the public's reaction to the news of the twins' birth. Another huge flaw in He's experiment was his blindness before the implantation of the embryos to any possible side effects or off-target edits from the CRISPR-Cas9 system that may have affected each embryo. Before this study, Jiankui He had no prior experience in medicine; in fact, his Ph.D. was from the Department of Physics and

Astronomy at Rice University. Following this scandal, He was placed in prison for several years for his part in the HIV prevention study. His example should not be followed, as this controversy was a massive setback for the field of germline gene editing that has affected the ability of scientists to conduct research years later.

CRISPR-Cas9 Mechanism

Understanding the mechanism and workings of CRISPR-Cas9 technology is essential in determining whether it is ethical to use in medicine. The CRISPR-Cas9 system has been nicknamed the "molecular scissors" for its ability to induce double-stranded breaks in a target stretch of DNA. Cas9 is a type of protein called an endonuclease and is the mediator of this DNA breakage. Cas9 has two different domains that, together, cause a double-stranded break in the DNA. Both the RuC domain and the HNH domains each cut the DNA, with each domain responsible for cutting a specific strand; the RuC domain cuts the strand that is non-complementary to the spacer sequence, and the HNH domain cuts the complementary strand. Immediately after this induced break, the DNA is restored in one of two processes: non-homologous end-joining (NHEJ) or homology-directed repair (HDR). NHEJ involves arbitrarily adding or removing nucleotide bases (intels) at the site where the DNA was cut. HDR uses the homologous region of the unedited DNA strand as a template to correct the damaged DNA, which is seen as a seemingly flawless method of repair. In the case of gene editing, if a template is present, the DNA will be repaired using NHEJ. Without a template present, HDR is used. One of the barriers to expanding the applications of CRISPR-Cas9 is finding a way to ensure HDR is always used to restore the DNA since it is much less likely to cause potentially

harmful errors.

CRISPR-Cas9 cannot function without two crucial types of RNA, one named crRNA, or CRISPR-associated RNA, and the other named tracrRNA, or trans-activating CRISPR RNA. crRNA's purpose is to steer proteins to the target portion of the DNA, while tracrRNA is involved in the processing of crRNA and the actual breakage of the DNA by Cas9. Without both crRNA and tracrRNA, CRISPR-Cas9 cannot locate the appropriate place to induce double-stranded breaks into the DNA. Together, crRNA and tracrRNA are often referred to as single guide RNA (sgRNA). The viral vector used to deliver CRISPR-Cas9 is called an Adeno-Associated Virus (AAV). AAV is a single-stranded DNA virus that has the ability to insert a gene into the genome. Some of the advantages of using the AAV to deliver CRISPR-Cas9 are that it can induce long-term effects by inserting genes, and it can bond to many different types of receptors in the body, making it ideal for targeting a variety of disorders and many parts of the body. A new method utilizing the CRISPR-Cas9 system, called prime editing, causes tiny cuts in the DNA rather than double-stranded breaks. This method can be used to edit single nucleotide bases and has fewer aversive side effects than the typical gene editing methods. Prime editing is up and coming and definitely needs more fine-tuning before it can safely be used to treat human disorders. Germline gene editing is achieved by injecting CRISPR-Cas9 nucleases programmed to target specific DNA sequences, plus their RNA counterparts, into zygotes. One of the most significant problems with the CRISPR-Cas9 technology is that Cas9 can cause off-target mutations. In other words, rather than just inducing the planned edit, Cas9 can cause unwanted edits to the genome in other locations, raising questions about its efficiency and safety concerning potential human subjects. On the other hand,

many genetic disorders are caused by a single gene, so germline genetic editing may be the only way to find a cure for such disorders.

Ethical Concerns

As previously mentioned, most people believe germline gene editing is a tool with great potential for success but also great potential for disastrous results. Many causes for concern must be addressed before germline gene editing can become legalized internationally and a common practice in medicine. A significant component behind the ethics is the benefit-to-risk ratio, and the higher this value becomes for CRISPR-Cas9, the more likely germline gene therapy will be able to be utilized in clinical trials and approved as a treatment for diseases. As of 2018, over sixty-one ethics reports from over fifty countries have been released regarding germline gene editing. Of those countries, many felt it was too soon to pursue the practice of heritable mutations, with 54% expressing germline editing should not be allowed, 30% feeling ambiguous in their opinion, 11% thinking germline editing has a possibility of being allowed in the future, and 5% open to further exploration right now (Brokowski, 2018). Three of the main umbrellas of ethical concern are safety, the inability to obtain consent from future generations, and the possibility of the elite using the CRISPR-Cas9 technology to create “designer” babies.

Safety

Like any up-and-coming medical technology, CRISPR-Cas9 has some safety limitations that must be addressed before it becomes a commonplace treatment in the medical field. First and foremost, the biggest safety concern regarding the CRISPR-Cas9 system is that it can cause off-target edits (OTEs). OTEs are breaks in the DNA caused by CRISPR-Cas9 that were not

intended to be made. This is an obvious fault, as these OTEs are not predictable and, at present, not preventable. Their effects vary, depending on what genes are mutated, but may pose a significant risk to the organism whose genome is altered in an unknown way. Many scientists are researching ways to prevent this, which would be a huge step forward in approving future clinical trials using germline gene editing therapies. Present technology is not advanced enough to detect every ill-causing mutation, so embryos with critical diseases or disabilities may be brought to term, causing unnecessary pain and suffering. The less-than-ideal accuracy of CRISPR-Cas9 is the culprit of OTEs, so understanding what controls the system's level of accuracy would decrease this risk of OTEs and eventually make OTEs a negligible effect. Even breaks in the correct locations in the genome can cause problems; for instance, cells may develop cancer-like tendencies. CRISPR-Cas9 is also much better at inducing breaks in the DNA than inserting new genetic material. Specific to germline gene editing is the lack of knowledge regarding the effectiveness of gene editing in embryos, and there is a greater risk of inducing mutations that can wreak havoc down the familial line. However, the caveat to this fact is that the only way to increase this knowledge is to perform clinical trials and studies using the CRISPR-Cas9 technology, which is not legal in much of the world. Without the legalization of germline gene editing, its vast potential may remain untapped. A more ethical way to begin pre-clinical research on embryos is to enforce regulations that prevent these embryos from being brought to term. Therefore, studies can still provide valuable knowledge about germline gene editing and its effects without crossing ethical boundaries. In the future, this knowledge gained from pre-clinical research could be applied to clinical trials. Much of the backlash against germline gene editing seems to stem from a fear of the unknown, which will remain unknown unless clinical trials are permitted to commence. One of the biggest setbacks regarding clinical

trials and germline gene editing was the fiasco Jiankui He created when he used the technology to create edited embryos that were brought to term. Once a better baseline of knowledge is established, another step would be to use germline gene editing on embryos tested and known to have severe genetic deformities that should not be brought to term. As of 2015, 29 out of 39 countries surveyed banned germline gene editing from taking place for the purpose of replication (Ishi, 2015). The NAS/NAM Committee compiled a list of recommendations about human germline gene editing in 2017 (National Academies of Sciences, Engineering, and Medicine, 2017). Several of these recommendations focus on the safety of the technology, one example being "ongoing, rigorous oversight during clinical trials of the effects of the procedure on the health and safety of the research participants" (Coller, 2019, p 300.). Another argument supporting germline gene therapy is the number of healthy babies that could be born with its utilization in medicine. In-vitro fertilization (IVF) and pre‑implantation genetic diagnosis (PGD) are technologies that have allowed thousands of families struggling to get pregnant the opportunity to have a child. Many of these people also utilize IVF for the ability to test and choose embryos that have not inherited a specific harmful mutation. Germline gene editing has the potential to create more viable embryos that could be used for implantation, therefore increasing the success rate of IVF-mediated healthy births. This technology could also be used to decrease the incidence of miscarriages by ensuring embryos do not have any fatal mutations before development even begins to occur. Before CRISPR-Cas9 can be used to create heritable edits in the human genome, the safety and efficacy of the technology must be concrete.

Consent of Future Generations

Any study conducted on human participants, from completing simple surveys to partaking in intrusive procedures, must obtain informed consent from each person who participates. However, when it comes to germline gene editing, this is a gray area since the embryos whose genomes are edited have no say in the matter. The parents-to-be could provide this informed consent for that particular embryo, but who is consenting to the mutations that can now be passed down through several generations? It can also be argued that their consent is not required since embryos are not yet people. If embryos can be used for research, it may bring substantial scientific advances that cannot be found using other methods. However, this process may bring about lethal consequences for embryos, so ethics is a considerable factor in the debate about embryonic consent. The fate of embryos in IVF is also up to the parents-to-be and the doctors to choose what embryos to implant into the mother. On the legal side of things, a trusted person could be chosen to take on the responsibility of consenting to the genetic editing of embryos since the embryos cannot advocate for themselves. The debate about consent comes down to the fact of whether or not people alive right now should have the ability to dictate editing genomes of people who have not been born yet. Genes encode all the pieces that together program what makes every person a unique individual, plus social forces help to shape this person into who they will become. Changing this programming removes that individual's autonomy, which could lead to problems with their sense of self and self-identity. Social forces have shaped cultures internationally for thousands of years, with examples such as the domestication of animals and the invention of the telegram. Germline gene editing can be viewed as a social force, so its lasting effects may prove to be positive ones, such as eradicating disorders like cystic fibrosis or engineering a drought-resistant potato crop. A great example is the invention of the smartphone;

the consent of future generations years after this invention did not need to be considered, so germline gene editing technology should not be treated any differently. Smartphones have brought forth a plethora of functions into the hands of individuals that once required desktop computers- something nobody dreamed of a few decades ago. Germline gene editing may become one of those breakthroughs in the scientific and medical communities. One thing that will make this ethical consideration less concerning is increasing the benefit-to-risk ratio of CRISPR-Cas9-mediated germline gene editing. If the benefits outweigh the negative consequences of the procedure, germline gene therapy would be one step closer to being approved for clinical trials and eventually being considered an appropriate treatment method.

"Designer" Babies

Imagining a world full of genetically engineered people is an eerie thought, but it may be our future one day. One of the main concerns around the legalization and mainstream of germline genetic editing is who can use the technology. Some fear that if it falls into the hands of the wealthiest elite, it will be utilized to create a group of "designer" babies with whatever traits they please. This could be as simple as blue eyes or as complex as a near-perfect IQ- the limits are uncharted. Professional sports teams could be entirely comprised of people engineered to be talented in a particular event, and scientists researching at the top universities could be the same. This would bring up a variety of issues, from discrimination in the workplace to scholarships at top universities, and especially social equality. These people engineered to be perfect may receive preferential treatment, leading to segregation between those who are genetically engineered versus those who are not, reminiscent of horrible periods of eugenics, such as the Holocaust or forced sterilizations in the United States, that should not be repeated. A social

divide like this can wreak havoc on a society. Another critical relationship that this scenario could affect is the bond between parents and offspring. A child genetically programmed to be the "perfect" son or daughter could drive a wedge between the child and parents and cause the child to feel like they will never be good enough. Another possibility, almost the opposite of "designer" babies, is governing bodies using CRISPR-Cas9 and germline editing to produce a population of individuals with traits that would make them easy to control and less likely to revolt; in other words, a workforce made up of controllable individuals. This idea is almost more frightening than the "designer" babies and crosses many ethical boundaries. Governments could also shift their focus from weapons like bombs and technology to engineered human soldiers- a sort of arms race (Coller, 2019). However, we still are not advanced enough in our knowledge of genetics to understand what genes encode each and every trait, so these possibilities of "designer" babies, a population of enslaved people, or human warriors are far-fetched. Several legal and ethical roadblocks would prevent these types of people from getting ahold of CRISPR-Cas9 and using it for corrupt purposes without being approved by several groups. Although many people are wary of CRISPR-Cas9 mediated germline gene therapy being used for purposes other than curing disease or research, just as medical procedures are used to enhance humans, germline gene editing could be used similarly. Plastic surgeries and Lasik eye surgeries can be performed simply to change someone's appearance to their liking or correct their vision- neither are technically medically necessary, but they are both prevalent procedures. Germline gene therapy could eventually eradicate genetic disorders and allow a family to be engineered to all have red hair, neither of which is medically necessary but could improve their quality of life. In this way, genetic editing could be seen as a form of free expression, one of the freedoms United States citizens enjoy. Drawing the line between medically necessary procedures

and human body enhancements is also challenging. For example, a germline gene editing procedure that treats a condition such as diabetes could be used for a patient without diabetes to aid in weight loss that is not medically necessary. Even so, both uses of the treatment improve quality of life, so this argument is not as strong as some others against germline gene editing.

Preclinical & Clinical Research

Since its discovery, several successful clinical trials have been published using CRISPR-Cas9 via somatic gene therapy; for instance, a recent study conducted in Japan showed gene therapy significantly improved symptoms of aromatic l-amino acid decarboxylase deficiency (Kojima, 2019). However, because most of the scientific community believes germline genetic editing should not be allowed for the foreseeable future, very few legitimate studies have been conducted using it thus far. Because of the ethical debate, no recent legitimate clinical trials have utilized germline genetic editing. However, pre-clinical research, or that conducted on animal models or embryos rather than humans, is a way to study germline genetic editing with fewer ethical standards to abide by.

One exciting study (Huai, 2017) hoped to cure mice bred to have the X-linked disorder hemophilia B. Hemophilia B was a perfect candidate to target with germline genetic editing since a single mutation in the F9 gene causes the body to produce nonfunctional factor IX, which is essential for blood clotting. Patients who suffer from severe hemophilia B have lifelong bleeding issues that, in severe cases, can even lead to death. Results from the somatic gene editing portion of the study showed that the mice in their Cas9-treated group improved their condition from a severe case of hemophilia B to a moderate or mild version of the disorder, plus an increase in

their percentage of factor IX. Researchers concluded from the success of the first portion of the study that it is necessary to explore the possibility of using Cas9 via germline gene therapy to combat hemophilia and prevent its heritability in future generations. This part of the study focused on the safety and accuracy of three types of germline gene therapy. They found that Cas9 protein-mediated treatment was the best method in terms of embryo integrity and also caused more effective gene correction. Mice in both Cas9 treatment groups were found to have higher levels of factor IX than the mice who had their F9 gene knocked out. Therefore, the germline gene therapy successfully treated hemophilia in the mice. This preclinical experiment provides solid evidence supporting the future use of CRISPR/Cas9 in vivo and ex vivo in germline gene editing and medicine.

Conclusion

CRISPR-Cas9-mediated technology holds great promise in medicine, especially regarding germline gene editing. Before germline gene therapy can be approved for use in clinical trials, however, some ethical concerns must be taken into consideration. It is essential to consider all of the ethical dilemmas editing the germline brings to light, from fear that it will be used for corrupt corporate gains to the safety of the patients it will be used on. I believe the most significant obstacle to the legalization of germline gene editing is the gaps in knowledge about how to increase the accuracy of CRISPR-Cas9. Once the concerns regarding off-target edits and the perfection of the technique used to edit the germline have been addressed, there is a much stronger argument to begin clinical trials targeting single-gene disorders like cystic fibrosis and sickle-cell anemia. The first clinical trials should utilize embryos deemed unusable for IVF, assuming no ethical boundaries will be crossed; these embryos should not be brought to term but

will be an excellent resource for researchers to improve the precision of the CRISPR-Cas9 system. Before the technology can be translated into treatments and cures for genetic disorders, it must be proven successful and safe for use in humans. Safety is the biggest priority; the other ethical concerns may become negligible if germline gene therapy shows enough promise in curing disease. The capabilities of CRISPR-Cas9 are vast, but its biggest impact will be in medicine. There are a plethora of disorders other methods simply cannot treat, and this technology will open the door to a future world without millions suffering from genetic disorders. Although it is nearly impossible to resolve all of the ethical issues surrounding germline gene editing, pre-clinical research is an excellent place to begin, and this research will hopefully lead to successful clinical trials in the future.

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