Germline Gene Editing: Ethical Considerations for Safe Clinical Research

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Abstract

Human genome editing has become a popular subject for clinical research. This research is becoming widely performed as technologies develop to make genome editing an easily accessible task. One such method uses clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) to cut out portions of DNA from a genome, with the possibility of pasting in a different DNA sequence. Using CRISPR-Cas9 to edit the genome of human embryos may one day replace current practices used to ensure genetically related offspring absent of genetic disorders, such as preimplantation genetic diagnosis (PGD) combined with in vitro fertilization (IVF). However, embryonic genome editing remains both ethically and practically controversial for a multitude of reasons, which will be explored and explained in the following text, along with possible solutions.

In November 2018, Dr. He Jiankiu of the Southern University of Science and Technology in Shenzhen, China announced that the first genetically modified babies had been born. The non-identical twin girls, nicknamed Nana and Lulu, are a product of two embryos produced by a willing couple. Their father is HIV positive and their mother is not. Besides the minimal amount of disclosures and information released by Dr. He Jianku and his team, who are still under investigation and cannot be considered completely reliable due to the lack of reliable scientific procedures followed over the course of their work, little is known about the experiment. The announcement preceded the commencement of The Second International Human Genome Editing Summit by a mere day (Greely, 2019). This Summit brings together scientific representatives, policymakers, and other experts from around the world to discuss genome editing from a variety of perspectives, although no global policies exist regarding the subject.

DNA is genetic material in every living organism and creates characteristics that make each person unique. Scientists in recent decades have been conducting more and more research on modifying DNA sequences, a process known as gene editing. This is the manipulation of a living organism's DNA by either deleting, replacing or inserting a genetic sequence that can correct or treat a genetically influenced trait or disorder. Current research projects involving common diseases such as diabetes, cancer, and HIV have shown promise in laboratory settings. However, recently there has been debate as to whether or not it is ethically permissible to continue researching genome editing for a variety of reasons. One of the primary causes for hesitation is the significant incidence of mistakes that still occur in laboratory trials of the technology. Another is the sourcing of the embryos; the use of viable human embryos for research is ethically questionable to many, though there are other

alternatives. A final concern is the potential for abuse of the technology. Once genome editing becomes widely available, it will be difficult to distinguish between its use for clinical applications and non-medical enhancement purposes which can create a slippery slope toward discrimination. While these ethical considerations are appreciable, the tremendous benefits that could be gained from germline engineering technology are substantial enough that the research must continue. However, in order to address the ethical considerations involved, specific concessions must be made including the use of tripronuclear zygotes for further research and the implementation of strict regulation of the testing and the eventual application of the editing.

Genome editing, specifically using the CRISPR-Cas9 system, has the potential to greatly advance gene therapies and the treatment of hereditary diseases. There has been a general increase in the number of publications referencing the use of gene editing techniques using zinc finger nucleases (ZFNs), transcription activator like effector nucleases (TALENS), and CRISPRs since the early 2000s. Of these methods, the CRISPR-Cas9 system was reported with the most significant increase of use from 2013-2016 (Pinez-Pinera et al, 2016). Although still in the early stages of development and use, CRISPR-Cas9 techniques have already proven less costly, with easier access to the materials needed to perform the process, than other techniques, leading to an increased possible output of genetic research (Cribbs et al, 2017). It has become preferred for research and development due to its superior specificity, efficiency, accessibility, and versatility (de Lecuona et al, 2019. CRISPR is able to precisely target and exact region of DNA down to a specific base pair, leading to a lower chance of changes to unintended genes. The technique is also easily applied to different organisms and different tissue and

cell types. These characteristics make CRISPR technology simple, more precise in application, and more cost-efficient than the others.

Human genome editing has the potential to address a wide variety of genetic diseases and has shown promising results of doing so in trials, and this is one of the primary arguments in favor of continuing research. Approximately 7.9% of babies are born with a severe birth defect that is genetic in origin or has a genetic component (Christianson, Howson & Modell, 2005). This amounts to about 10 million children each year carrying the burden of disease, and many of these diseases could be addressed with therapy using genetic engineering to ameliorate the impact of the disorder. Gene editing is most widely viewed as a potential solution to monogenic diseases (single gene Mendelian-style disorders) such as Huntington's disease, Tay Sachs disease, or Cystic Fibrosis. Medical professionals have defined about 7000 such diseases so far (Christianson, et al, 2005). However, it has the potential to be used as a therapy to treat some of the effects of more complex polygenic disorders such as schizophrenia, that cannot be addressed using any other method of treatment. Applications of the technology have been developed in trials involving diseases ranging from sickle cell anemia and thalassemia to HIV and a variety of cancers (Bronkowski & Adli, 2019). Clinical trials targeting human diseases in the last several years have shown improving results in the accuracy and efficiency of the CRISPR technology. Trials on human stem cells *in vitro* (outside the organism, in a test tube) have tested Barth syndrome, an X-linked genetic heart disease in humans, and have been successful in identifying the causal mutation in a wide variety of diseases including muscular dystrophy, Parkinson's disease, and Huntington disease. In tests involving mice, rats and monkeys carrying a variety of human diseases, the use of *in vivo* (inside the living organism) CRISPR-edited cells have also had incredible success. In these cases, edited cells were reintroduced to the organism, after which they were seen to

remedy the disease. Other potential therapies that have shown promise in animal trials using CRISPR technology involve the recognition of cancer cells for certain types of cancer in mice and the blockage of continual infection of T-cells in individuals infected with HIV (Cai, Fisher, Huang, & Xie, 2016). These examples indicate the potential of genome editing to address genetic diseases and benefit a wide variety of people and give reason for the continuance of research to develop and perfect the technology.

Another compelling case for continuing genome editing testing is that applying these technologies may also lead to more favorable options than PGD and IVF treatments. PGD/IVF combinations lead to a large number of viable embryos being discarded with each pregnancy (Steffann et al, 2018). This can be attributed to a surplus of embryos needed to increase the odds of creating a viable embryo, with the destruction of embryos found to carry affected genes. Being able to edit the genome of an affected embryo would mean that a smaller specimen number would be needed to begin with, decreasing the number of viable embryos destroyed, which has been an ethical concern.

However, there are several risks involved with the genome editing process that need to be considered before it is applied in a clinical setting. One commonly debated issue is the safety of such genetic modification and how these concerns can be addressed to allow its use in a clinical setting. The main concerns involve off-target mutations and mosaicism. Off-target mutations in gene editing are when unintended mutations occur after the editing process is complete. Consequently, these mutations have the potential to cause diseases that can be inherited by the following generations (Savulescu, 2015). These random mutations are hard to predict because currently, the software developed specifically for detection still misses many of these instances. Mosaicism is when an embryo carries both edited and unedited cells. An organism exhibiting signs of mosaicism has the potential to

develop other diseases. Mutations in areas such as tumor suppressor genes could lead to leukemia because instead of protecting the body it could alter the genes into causing harm (Carroll, 2019). Because of these safety issues, many researchers discourage the implementation of gene editing technology until improvements dramatically decrease the incidence of such mistakes. This led many to question the reliability of the CRISPR process. However, current research shows promise; experiments conducted on monkey embryos resulted in no off-target mutations. Similarly, in a laboratory trial on human embryos, only two out of six embryos injected with Cas9 exhibited off-target effects (Guo, 2015). These concerns have left many researchers feeling apprehensive when approaching this topic, yet, abandoning this topic altogether would be redundant as this research has the potential to solve many disease-related issues. Research that is guided by caution, reason, oversight, and transparency can alleviate the safety concerns associated with gene editing.

An additional issue surrounding the ethics of genome editing stems from the process of experimentation and the sourcing of the specimens used to conduct research. Many of the experiments conducted on animal specimens (mostly mice) have proven successful and insightful. For example, in studies conducted on mice with the intention to eliminate cataracts, researchers were able to decrease the number of off-target mutations that occurred when CRISPR technology was utilized to remove genes responsible for cataracts (Wu et al., 2013). However, for similar testing of this technology to be ethically permissible in humans, the testing must become far more precise and has to graduate from animal to human specimens, otherwise it is still merely hypothetical. The idea of creating and testing on human embryos is controversial, with most people arguing in defense of the embryos, citing that one could inflict pain at later stages of development and that the discarding of viable human embryos

is immoral and therefore unthinkable. Unfortunately, for researchers to gain a better understanding of how genome editing will affect the complex human DNA, research on human embryos is vital.

The issue of obtaining human specimens for research can be addressed through the use of tripronuclear zygotes. These are embryos created for in vitro fertilization that are formed from eggs fertilized by more than one sperm cell (normal zygotes contain only one). Post-implantation, they do not develop into viable fetuses and are typically discarded in clinics. Use of these embryos would address the concerns of those in argument with the sources of specimens since these embryos are already in existence (not created with the sole purpose of scientific experimentation), they are nonviable, and they would be otherwise discarded in clinics. Justifiably, current legal tests performed on embryos include PGD. This process involves the removal of two to eight cells from an embryo which has the potential to be even more detrimental than the proposition of gene editing on these embryos (Savulescu et al., 2015). If a practice with a similar effect on embryos is currently legal, the question is, why isn't gene editing? Currently, there have been a few studies that have illustrated the efficacy of genome editing through testing on tripronuclear zygotes. For example, in a study conducted using tripronuclear zygotes, researchers utilized a different mechanism in efforts to reduce the number of off-target mutations occurring, which has been another looming concern within the gene editing spectrum (Zhou et al., 2017). Without their ability to test on embryos, researchers would never have been able to determine that it was, in fact, possible to achieve such a small or nonexistent amount of off-target mutations. With the requirement that further testing be limited to these nonviable tripronuclear zygotes, ethical concerns regarding the sourcing of embryos can be addressed.

Most agree that restrictions need to be made and guidelines put in place regarding research and the potential implementation of genome editing, though many countries do not have any and those

that do are ambiguous and minimal. In December 2015, nearly 500 scientists, ethicists, legal experts, and advocacy groups from all over the world came together in Washington DC at the first international Summit on Human Gene Editing to discuss and establish guidelines for the use of gene editing in humans, both before and after birth. This was a collaboration of a much more diverse group of people than previous attempts at convergence on the subject. It established one important point: to inhibit germline gene editing pre-birth, or embryonic gene editing (Reardon 2015). Where this line is ultimately drawn will define the future actions of scientists like Dr. He Jiankui. However, scientific collaboration may not hold with regards to application, as was proven by Dr. Jiankiu's experimentation. A consensus among scientists does not equate with law, nor does it carry consequences upon breaking them. In order to prevent abuse in genome editing research, legislation must be passed to regulate clinical experimentation. Such legislation could be created and carried out by individual countries. At this point, current policies between countries either differ dramatically, are extremely ambiguous or do not exist at all. For example, most of Africa, parts of the Middle East, Europe, and South America, are either ambiguous towards the gene editing of human embryos or have no mention of such application in their laws, compared to Canada and Australia who has upright ban on human gene editing (Araki et al, 2014). Without rules and regulations or defined penalties for breaking those rules, the abuse of the technology such as CRISPR in human gene editing cannot be regulated. Before Dr. He Jiankui's experiments, there was existing legislation in China which prohibited the editing of human embryos. These laws, however, did not describe any penalties (Cyranoski, 2019). Dr. He Jiankui's work is considered abuse from both a legal and ethical standpoint and is still under investigation. Scientists in many countries objected to his work, declaring it unsafe and unethical. Soon after the announcement of his experimentation, penalties were put into place by

the Chinese government to tighten the laws and further set regulations (Cyranoski, 2019). Because of the substantial potential for abuse and widespread agreement that there is a need for ethical guidelines, research and clinical application of genome editing should be addressed in three different ways, among which regulations at a national level are the most effective, such as China's inclusion of penalties in legislation for abusing gene editing on humans, voluntary self-regulation, and the least effective, public consultation (Charo, 2016) . China's blindness before the regulation was violated shows that if the laws at a government and national level are not fully defined, major flaws in addressing the issues may arise. Voluntary self-regulation, or in other words people from different backgrounds coming together to establish a regulatory system, is the most effective way to stop the abuse of gene editing on humans. For example, 1975's Asilomar Conference, which provided regulations for the use of recombinant DNA technology (Charo, 2016), and aforementioned 2015's Summit on Human Gene Editing. These voluntary regulations not only get adopted as government laws, but also, keep rogue researchers in check by limiting collaboration and resources (Charo, 2016). In conclusion, initiating the collaborations of both scientific and public communities to produce well defined rules and regulations, and later adopting those rules in legislation by the government, is the best way to set boundaries in human genome editing.

Genome editing has the potential to treat many genetic diseases. However, before research should be allowed to continue and the practice accepted in clinical settings, there are a number of ethical concerns that need to be addressed. One concern is the safety of the process which can be improved by conducting and perfecting further research on human specimens. Ethical concerns behind the use of human embryos can be alleviated by the use of tripronuclear zygotes for laboratory research. Finally, strict regulation and laws can prevent the potential abuse of germline editing for

non-medical use. With these adjustments, genome editing can shift its status from ethically questionable to acceptable and many diseases may be eradicated from existence. A world where the cure to diseases such as Huntington or Tay Sachs Disease lies within a few edits in a petri dish. The average lifespan of humans could increase and the ten million children per year that are currently enduring the trials of genetically related diseases would no longer have to suffer.

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