Editing of The Human Genome Using CRISPR and The Ethical Implications of Doing so.

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Abstract

The powerful genetic engineering technology CRISPR/Cas9 has been used extensively in the last decade for agricultural advancement and human disease research. Clinical trials have successfully treated monogenic diseases in humans and transgenic livestock have been reliably produced using embryo editing. To the idea, integration with human IVF, has the potential to eradicate human genetic disease and provide means of human enhancement. Ethical questions surround IVF procedure, safety of CRISPR technology, connotations of editing an embryo, and the effects when editing humans would have on society. In potential uses of gene editing, as well as a variety of ethical concerns, this review aims to understand the ideal way for the world to proceed with research and implementation of the technology. The research of human somatic cell and embryo editing should proceed, but it should be done responsibly and slowly, with regulations being established to ensure the wellbeing of society, safety of patients, and equal availability of benefits.

Introduction

Genetic engineering has been a major field of research since the late twentieth century. Initially, research was focused on improving crops and livestock with basic techniques. In the twenty-first century, development of programmable endonucleases allowed more sophisticated editing of livestock embryo genomes (Navarro-Serna et al. 2020). In 2012, the programmable endonuclease system CRISPR/Cas9 was developed as the fastest, most precise, cheapest, and most efficient gene editing system ever (Jinek et al. 2012; Redman et al. 2016; Navarro-Serna et al. 2020). Since 2012, CRISPR technology has been used extensively for editing zygotes of

animals using in-vitro techniques, and for human heritable disease therapeutics by somatic cell editing (Redman et al. 2016; Navarro-Serna et al. 2020; Abdelnour et al. 2021; Frangoul et al. 2021). In Vitro Fertilization (IVF) is also a common technique used for human reproductive aid, and greatly lends itself to integration with CRISPR embryo editing. However, IVF itself is known to be invasive for its patients, and there are a plethora of ethical concerns associated with the genetic engineering of the human germline or human embryos (Niemiec et al. 2020; Romito 2022; Choe et al. 2023; Steynberg 2023). We aim to investigate what ethical considerations need to be made before the potential of genetic engineering. By outlining historical and current uses of gene editing technology and a variety of ethical standpoints regarding future human germline editing, we aim to bring clarity to the necessary measures which must be taken before this technology becomes commonplace.

Discussion

Introduction to In Vitro Fertilization

Human IVF involves collection of oocytes from a donor, production of embryos outside the body using male gametes, and reimplantation into the mother or surrogate (Romito 2022; Chloe et al. 2023). The window of time between fertilization and implantation lends itself to most genetic engineering procedures (Redman et al. 2016). This stage of IVF is how edits are most commonly made to livestock embryos. The potential for human editing is also apparent. Even excluding the potential of genetic editing, widespread IVF could pose ethical risks, especially towards women. Oocyte procurement is a particularly invasive procedure, and if

embryo demand for research increases, it could become exploitative toward women (Niemiec et al. 2020; Romito 2022).

Progression of Programmable Endonuclease Genetic Engineering Technology

Genetic engineering or gene editing involves intentionally making changes to the nucleotide sequence of an organism's DNA. By far the most powerful type of gene editing technologies are programmable endonucleases, which cleave DNA at a predetermined or "programmed" location. The first programmable endonuclease technology was zinc-finger nucleases (ZFNs), which were used to create transgenic animals (Navarro-Serna et al. 2020). ZFNs were followed by transcription activator-like effector nucleases (TALENs) which had higher specification, higher efficiency and lower cytotoxicity (Navarro-Serna et al. 2020).

These technologies have now been largely replaced by the more precise, faster and cheaper CRISPR/Cas9, a programmable endonuclease discovered in 2012. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR associated proteins (Cas) refer to RNAs and proteins present in some bacteria (Jinek et al. 2012). The most commonly used Cas endonuclease is Cas9, derived from Streptococcus pyogenes (Navarro-Serna et al. 2020).

Cas9 complexes with CRISPR guide RNAs (gRNAs) bound to target DNA, and enzymatically induces a double stranded break (DSB) in the DNA (see Figure 1) (Redman et al. 2016; Ryu et al. 2018; Navarro-Serna et al. 2020).The DNA is edited when the DSB is repaired, most commonly by non-homologous end joining (NHEJ) or homology directed repair (HDR) using either homologous or sister chromatids (Ryu et al. 2018; Navarro-Serna et al. 2020).

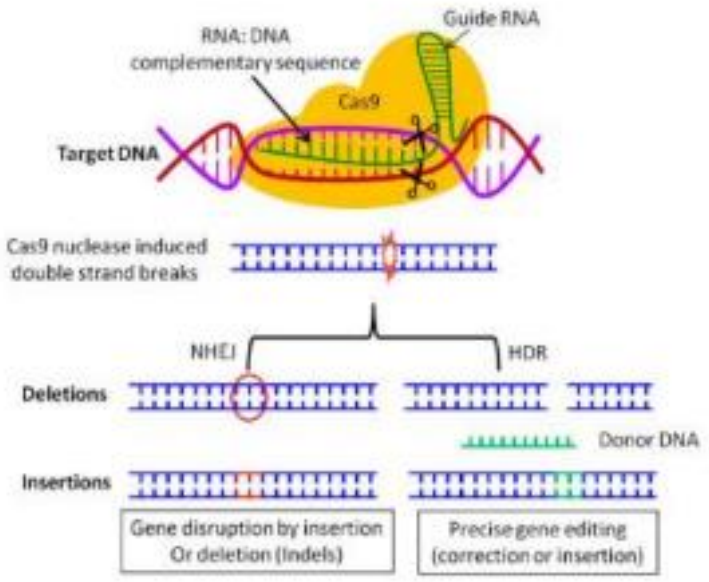

Figure 1. Illustration of the gRNA and Cas9 complex with double stranded DNA. gRNA binds to double stranded DNA by complementary base pairing at the specific site. Cas9 endonuclease induces the double stranded break and it can be repaired and edited by NHEJ or HDR (Abdelnour et al. 2021)

A common use for this procedure is embryonic editing of livestock, especially attempting a complete gene knock-out. This was successfully achieved by Crispo et al. (2015) when ten lambs were born with a knocked-out myostatin gene and varying phenotype. The most common method of delivery is zygote or oocyte microinjection of CRISPR/Cas9 encoding mRNA or plasmid (Ryu et al. 2018; Navarro-Serna et al. 2020). The most common problem with zygotic microinjection is mosaicism, which is when an organism's cells do not all have identical DNA (Navarro-Serna et al. 2020; Niemiec et al. 2020). It should be noted that excessive animal

embryo editing research and the associated procedures do raise animal welfare concerns (Ormandy et al. 2011).

Use of CRISPR/Cas9 for Therapeutic somatic Cell Editing in Humans

The most common use of CRISPR technologies in human cells is for the research of treatment of monogenic heritable diseases by way of somatic cell editing. By 2016, pre-clinical research had been done to investigate possible treatments for cystic fibrosis (CF) and Duschenne's muscular dystrophy (DMD), among others. This early research focused on repairing a single locus and used cultured human stem cells or mice (Redman et al. 2016).

The most common heritable monogenic diseases in humans are two types of haemoglobinopathy, sickle cell disease (SCD) and β-thalassemia. Haemoglobinopathies are caused by mutations in the β-globin gene, which encodes the haemoglobin β subunit (Abdelnour et al. 2021). Clinical trials have determined that SCD and β-thalassemia can be treated by editing genes responsible for inhibition of fetal hemoglobin (HbF) production. Fetal hemoglobin does not get transcribed in adult humans, but by editing the genes of transcription regulator proteins (BCL11A or KLF1), HbF production can occur and replace unhealthy adult hemoglobin in haemoglobinopathy patients (Raposo. 2019; Abdelnour et al. 2021). For a typical treatment, researchers collect hematopoietic (blood producing) bone marrow stem cells and use CRISPR/Cas9 to target and deactivate the BCL11A locus. Upon reimplantation of the cells, they can propagate their edited DNA into daughter cells and produce healthy HbF.

In 2019, Victoria Gray was the first woman in the US to get genetic treatment for her SCD. Over 9 months after treatment, Gray showed none of her previous SCD symptoms and tests showed over 81% of her bone marrow was producing HbF (Steynberg 2023).

Another clinical trial in 2020 (see Figure 2) aimed to treat two transfusion dependant patients, one with SCD and one with β-thalassemia. Both patients had over 80% of alleles successfully edited and were both transfusion independent for over a year afterwards (Frangoul et al. 2021).

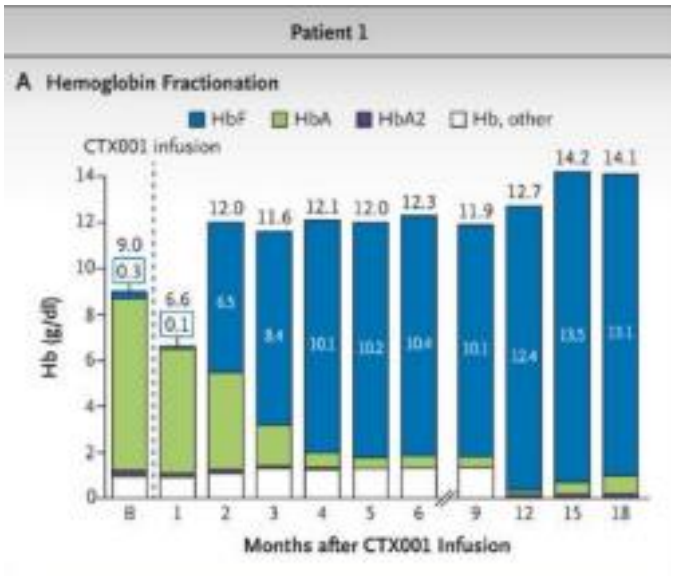

Figure 2. Amount of varying types of hemoglobin in the blood of the β-thalassemia patient prior to and following infusion of CRISPR/Cas9 treated cells. The KO of HbF production inhibitor gene was successful and the patient maintained healthy HbF levels for over 18 months. (Frangoul et al. 2021).

It should be noted that multigenic heritable diseases are significantly harder to treat since they require simultaneous edits at multiple loci (Redman et al. 2016). Other preclinical studies have researched treatment for atherosclerosis, retinoschisis, phenylketonuria, hereditary hearing loss and more (Abdelnour et al. 2021). All of the research looking at disease treatment above used somatic cell editing, but there is also potential in the next step: germline or embryo editing.

A Premature Milestone: Dr. He Jiankui's Experiment

The idea of a human being born from a genetically modified embryo was purely a thought experiment until 2018. Dr. He Jiankui, a Chinese scientist, used CRISPR/Cas9 and IVF procedure to edit two female embryos and then establish a pregnancy (Raposo et al. 2019; Niemiec et al. 2020; Singh 2021). Both girls were born and are the first humans to be born of edited embryos. Each embryo's biological father was an HIV carrier, and Jiankui's aim was to disable the CCR5 gene, which enables HIV infection. In theory this would protect both girls from their fathers' HIV (Raposo. 2019; Singh 2021). Testing of the girls' DNA revealed that the intended edit was not made in either girl, and in one of the girls, only one copy of CCR5 was edited at all. There was also evidence of mosaicism in both girls (Raposo. 2019).

The ethics of this incident are obviously dubious, and after it became public, the discussion on the topic greatly increased among ethicists and scientists (Raposo. 2019; Singh. 2021). At the time of Jiankui's experiment, Chinese regulations prohibited this kind of research, and he provided no evidence of legality or ethical approval (Raposo. 2019; Niemiec et al. 2020).

The experiment cannot be justified by labeling it a therapeutic intervention since the girls were not at risk of being born with HIV; sperm washing was done so that only healthy gametes were used in the procedure (Raposo. 2019; Niemiec et al. 2020). This lack of medical necessity, along with other critiques including the availability of alternative therapeutic methods, lack of ethical approval and the lack of informed consent obtained, label the experiment as reckless and unnecessary (Raposo. 2019; Niemiec et al. 2020).

Another issue presented is that changes to the CCR5 gene are known to cause higher risk of infection from certain viruses. The gene is also associated with some brain functions, and some speculate the edits could have enhanced the girls' intelligence. However, it is unknown if Jiankui was aware of this at the time of his experiment (Raposo. 2019). Furthermore, although there was no evidence of offsite edits presented in this case, the CRISPR/Cas9 technology Jiankui used was not sophisticated enough for human use, speaking further to the experiment being rushed (Raposo. 2019).

Despite this scary incident, the potential of CRISPR technology is too great to induce a complete ban on research, but we must proceed with caution. The incident was also arguably necessary to spark the depth of ethical discussion which resulted (Raposo. 2019; Singh 2021).

Understanding Ethical Issues

As gene editing has advanced, a large variety of ethical standpoints regarding human embryo editing using IVF have been presented. Many argue whether embryos created with IVF are considered human yet. Some believe they have moral value as soon as they are fertilized,

where others believe they are simply a cluster of cells (Lorenzo et al. 2019; Niemiec et al. 2020).

Much of the discussion is regarding whether research on human embryo editing (HEE) is worth the risks. The potential to protect a whole population from genetic disease is clear, (Redman et al. 2016; Niemiec et al. 2020), and some suggest there is a moral imperative to do so (Singh. 2021). Others point out that there are few cases where gene editing could protect a child from an disease existing IVF techniques could not (Niemiec et al. 2020; Singh. 2021). Moving past monogenic disease treatment, there is a general consensus that human enhancements should be avoided. Some use this to suggest against therapeutic research, saying that any intentional creation of an edited child is enhancement (Niemiec et al. 2020), or saying that allowing somatic cell editing research leads down a "slippery slope" toward eventual widespread human enhancement (Singh. 2021).

The safety of the CRISPR technology being used is also in question. While it is the best compared to any other technology, CRISPR/Cas9 is far from perfect, with low editing efficiency and high rates of off-target edits and mosaicism (Navarro-Serna et al. 2020; Niemiec et al. 2020;Singh. 2021; Raposo. 2019).

Taking the theoretical potential of human IVF embryo editing to the next level, we arrive at the concept of designer babies, where scientists could decide the traits of a child. Some say that this would violate the right to autonomy of future generations, and that there is a lack of informed consent from the unborn children (Niemiec et al. 2020; Singh. 2021). Furthermore, any technology that is differentially available across the world would foster social inequities and cause disparities that become permanently encoded into society (Singh 2020; Steynberg 2023).

Others say that widespread embryo editing would work to address inequities and level the playing field across the world (Singh. 2021).

The Approach to Take Moving Forward

While some argue human cell editing research requires minimal global regulation, the general consensus is that the variability in potential applications requires regulation from a large number of bodies across the world (Singh. 2021). It is the responsibility of influential global organizations to design strict regulations based on promoting well being, transparency, care and respect for patients, and international cooperation (Singh. 2021). This will protect humanity from threats to well being and social division. It is the responsibility of scientists to responsibly carry out their research slowly and responsibly, with regard for established regulations. By doing so, they can improve the efficiency, safety, and sustainability of genetic engineering technology so that its potential can be accessed ethically.

Conclusion

The discovery and development of CRISPR/Cas9 technologies has allowed rapid advances in genetic engineering of animals, and in research of gene therapy for human monogenic disease. There has been considerable success in creating transgenic animals by integrating CRISPR/Cas9 technology with IVF. Success has also been found in many clinical studies which use CRISPR to edit a patient's somatic cells, treating them for conditions like sickle cell disease. The first incident of human embryo editing was Dr. Jiankui's reckless

experiment, which sparked huge ethical discussion. The question of whether human gene editing research should continue was presented, given its potential and ethical barriers. Research should proceed under regulations which prioritize our well being and allow researchers to make progress safely.

Recommendations

Based on the research documented, this review recommends that gene editing research should remain a priority but should focus on optimization of current techniques and compliance with regulation rather than rushing toward groundbreaking developments.

References

Abdelnour SA, Xie L, Hassanin AA, Zuo E, Lu Y. 2021. The potential of CRISPR/Cas9 gene editing as a treatment strategy for inherited diseases. Front. Cell Dev. Biol. [accessed 2024 Mar 12];9. http://dx.doi.org/10.3389/fcell.2021.699597

Choe J, Shanks AL. 2023. In Vitro Fertilization. StatPearls Publishing. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK562266/.

Crispo M, Mulet AP, Tesson L, Barrera N, Cuadro F, dos Santos-Neto PC, Nguyen TH, Creneguy A, Brusselle L, Anegon I, Menchaca A. 2015. Efficient Generation of Myostatin Knock-Out Sheep Using CRISPR/Cas9 Technology and Microinjection into Zygotes; 2015; PLoS ONE. 10(8); https://doi.org/10.1371/journal.pone.0136690

Frangoul H, Altshuler D, Cappellini MD, Chen Y-S, Domm J, Eustace BK, Foell J, de la Fuente J, Grupp S, Handgretinger R, et al. 2021. CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. N. Engl. J. Med. [accessed 2024 Mar 15];384(3):252–260. https://www.nejm.org/doi/full/10.1056/NEJMoa2031054 doi:10.1056/nejmoa2031054

Romito K, Healthwise Staff. 2022 Jul 2. In Vitro Fertilization (IVF) | HealthLink BC. wwwhealthlinkbcca.https://www.healthlinkbc.ca/pregnancy-parenting/planning-your pregnancy/fertility/vitro-fertilization-infertility.

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. 2012. A programmable dual RNA–guided DNA endonuclease in adaptive bacterial immunity. Science (New York, N.Y.). 337(6096):816–821. http://dx.doi.org/10.1126/science.1225829.

Lorenzo D, Esquerda M. 2019. Map of ethical conflicts of the CRISPR-Cas9 gene edition technique. Medicina Clínica (English Edition). 153(9):357–359. doi:https://doi.org/10.1016/j.medcle.2019.03.018.

Navarro-Serna S, Vilarino M, Park I, Gadea J, Ross PJ. 2020. Livestock gene editing by one-step embryo manipulation. J Equine Vet Sci. [accessed 2024 Mar 12];89:103025. https://www.sciencedirect.com/science/article/pii/S0737080620301167. doi: 10.1016/j.jevs.2020.103025

Niemiec E, Howard HC. 2020. Ethical issues related to research on genome editing in human embryos. Comput Struct Biotechnol J. [accessed 2024 Mar 12];18:887–896. https://www.sciencedirect.com/science/article/pii/S2001037019305173. doi:10.1016/j.csbj.2020.03.014

Ormandy EH, Dale J, Griffin G. 2011. Genetic engineering of animals: Ethical issues, including welfare concerns. Can Vet J. [accessed 2024 Mar 12];52(5): 544–550. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3078015/

Raposo VL. The first Chinese edited babies: A leap of faith in science. JBRA Assist Reprod. 2019 [accessed 2024 Mar 12];23(3):197. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6724388/. doi:10.5935/1518-0557.20190042

Redman M, King A, Watson C, King D. 2016. What is CRISPR/Cas9? Archives of Disease in Childhood - Education & Practice Editing. 101 (4):213-215. doi: https://doi.org/10.1136/archdischild-2016- 310459.

Ryu J, Prather RS, Lee K. 2018. Use of gene-editing technology to introduce targeted modifications in pigs. J Animal Sci Biotechnol. 9(1). https://jasbsci.biomedcentral.com/articles/10.1186/s40104-017-0228-7. doi:10.1186/s40104-017-0228-7

Singh AN. 2021. Ethical Controversies and Challenges in Human Genome Editing. Int Med J. 28(2):134–137.https://ezproxy.tru.ca/login?url=https://search.ebscohost.com/login.aspx?direct=true&db=ccm&AN=149537843&site=eds-live&scope=site

Steynberg R. 2023. Designer Babies: Evaluating the Ethics of Human Gene Editing. Women Leading Change: Case Studies on Women, Gender, and Feminism. 7(1):49–60. https://journals.tulane.edu/ncs/article/view/3785.