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The Ethical Concerns of Using CRISPR-CAS9 for Gene Editing in Humans

Simran Mamik

Abstract: ***Hailed as a revolutionary game changer, CRISPR has been making the headlines for its multifarious utility from industry, agriculture to medicine. Its use particularly in the human context and in medicine has been a subject of debate with a section within the scientific community opposing the usage of this mechanism to modify human DNA, advising the supporters to be more cautious in their approach as it could have an irreversible far-reaching impact on humanity. This paper aims to investigate the various ethical concerns associated with this technology, calling for a more cautious approach.***

Keywords: CRISPR, gene editing, ethics, genetic modification, preventive health care

1. Introduction

Discovered by Dr. Jennifer Doudna and Dr. Emmanuel Charpentier, the term CRISPR-Cas9 genome-editing tool as we understand it today was first mentioned in their groundbreaking paper, "A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity" published in 2012. Their effort made them the first all-female team to win the Nobel Prize for Chemistry in 2020. However, it is important to note that there were others who had been talking about this mechanism earlier too. The discovery started an unprecedented race amongst companies and laboratories to patent this technology, hailing it as one of the most significant scientific breakthroughs of the century. In a medical context, CRISPR has shown potential to cure a range of genetic diseases including life threatening and disabling neurodegenerative disease, cancer, and blood disorders.

This genome editing technology developed from a bacterial adaptive immune system revises, removes, and replaces target DNA **[1]**, this mechanism has the potential to edit faulty genome and correct mutations that cause diseases showcasing its enormous potential for health care. CRISPR/Cas9 edits genes by precisely cutting DNA and then letting natural DNA repair processes to take over. The system consists of two parts: the Cas9 enzyme and a guide RNA **[2]**. The CRISPR-Cas9 functions like the genetic molecular scissors enabling medical researchers to edit parts of the genome by removing, adding, or altering sections of the DNA sequence. These molecular scissors guided and assisted by the chaperon RNA (gRNA can cut the two strands of DNA at a specific location in the genome so that bits of DNA can then be added or removed. Once the editing is done, cellular DNA repair pathways come into action, repairing the cut/addition. The two principal DNA repair pathways, used for gene editing are the non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ is exploited to render genes non-functional, while HDR is exploited to insert new genes or fragments of genetic material **[3]**. The diagram below is a graphic presentation to understand the basic approach of the process:

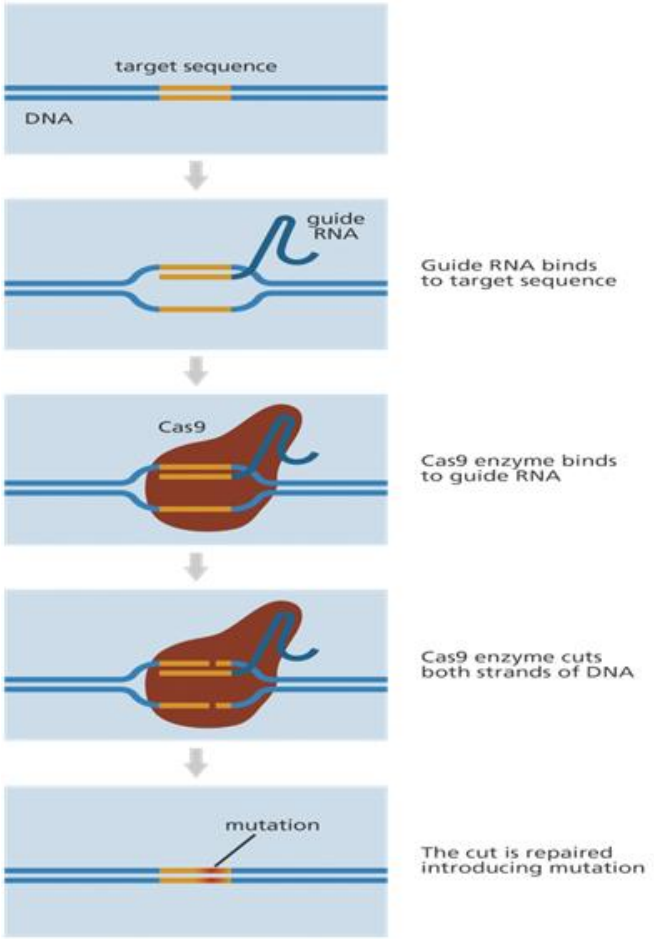

Diagram showing how the CRISPR-Cas9 editing tool works. Image credit: Genome Research Limited.

For decades, researchers have researched ways and means to manipulate DNA for health care and other purposes. "Cellular repair mechanisms to manipulate DNA through genome editing have the power to change the genome by correcting a mutation or introducing a new function **[4]** (Rodriguez, 2016), one that could work favourably in curing diseases caused by a genetic fault. CRISPR's impact and benefits of high accuracy, practicability and relatively low cost compared to other technologies in this domain has made it extremely popular.

Ironically the same benefits have triggered a raging debate around the ethics of using this technology in health care and

other fields as there is a growing mistrust that the relatively easy and inexpensive application of this technology may allow it to fall in the hands of unscrupulous elements who could use it for their short terms gains harming the long term interests of human kind and our planet, drawing international attention.

In 2015, a UNESCO panel of scientists, philosophers, lawyers and government ministers has called for a temporary ban on genetic "editing" of the human germline, calling for a wide public debate on genetic modification of human DNA. Subsequently, a report of the International Bioethics Committee (IBC) - "Updating its Reflection on the Human Genome and Human Rights" acknowledged its significance saying that "gene therapy could be a watershed in the history of medicine and genome editing is unquestionably one of the most promising undertakings of science for the sake of all humankind." . However, in the same report it cautioned "this development seems to require particular precautions and raises serious concerns, especially if the editing of the human genome should be applied to the germline and therefore introduce hereditary modifications, which could be transmitted to future generations" **[5]** arguing that the alternative would "jeopardize the inherent and therefore equal dignity of all human beings and renew eugenics." **[6]** and called for an international moratorium on it. This report validated the earlier ban on CRISPR experiments in over 40 countries, including from 15 across western Europe.

The news of the Chinese scientist He Jiankui, who claimed to have created gene-edited babies in his lab left the world community stunned and even more worried and ensured that this debate never died down. However, recent developments and studies on CRISPR genome editing to treat some rare diseases, are turning out to be promising mellowing down the sceptics and warming them towards accepting the changed reality. This is reflected in the key outcomes at the at the Francis Crick Institute in London, the UK Royal Society and Academy of Medical Sciences, the U.S. National Academy of Sciences and National Academy of Medicine, and UNESCO-The World Academy of Sciences held the Third International Summit on Human Genome Editing where the first two days were spent discussing somatic human genome editing, where the cells being altered are non-reproductive cells — as a result genetic changes cannot be passed on to future generations [7].

CRISPR is a useful technological tool that can help alleviate life threatening and disabling diseases; however it is fraught with ethical and technical challenges. If these challenges are not debated and controlled by accountable legal standards and framework, it could well become the proverbial Frankenstein's monster.

2. Ethical Concerns Associated with Crispr

2.1 Off-Targetside Effects

One concern is that CRISPR-Cas9 could result in mutations at locations other than those being targeted, which can potentially result in unforeseen consequences. This has set off an ongoing debate about its precise targeting and to what extent the occurrence of off-target effects matters (Eckerstorfer et al., 2019b, Zhao and Wolt, 2017) **[8]**. Off-target effects can be defined as unintended cleavage and mutations at untargeted genomic sites showing a similar but not an identical sequence compared to the target site (Modrzejewski et al., 2019) **[9].** Off-target effects consist of unintended point mutations, deletions, insertions inversions, and translocations.Researchers are worried that such unintended edits could be harmful and could even turn cells cancerous, as occurred in a 2002 study of a gene therapy [**10]**.

"If [CRISPR] starts breaking random parts of the genome, the cell can start stitching things together in really weird ways, and there's some concern about that becoming cancer," **[11]** Studies have found that off-target effects may appear in human stem cells (Suzuki 2014; Veres 2014) increases the likelihood that cancer cell lines will have higher rates of CRISPR/Cas9 unintentional targeting. This clearly shows that although CRISPR/Cas9 has ability to edit genes holds promise, the proves is not error free. The question that needs to be asked is who will pay a price for the trial and error involved in the learning process? According to the findings of the study "CRISPR/Cas9-mediated gene editing in human Tri pronuclear zygotes," of the 12 potential indels identified by this analysis, ten were on-target in all samples while two were off-target in samples A and C. By using PCR and sequencing, candidate off-target locations were further verified. Off-target effects can cause confounding variables in research experiments and can lead to potentially misleading and non-reproducible results, which can be a costly error in studies conducted on gene-editing **[12].**

Since there is usage of viruses to carry CRISPR, there is a possibility of it infecting multiple cells and not limits itself to the target cells only. This may lead to life threatening or disabling situations, where the procedure may for example end up editing muscle cells when the goal was to edit liver cells.

"Off-target effects can happen when there are more than three mismatches between the target sequence and the 20 nucleotides of gRNA. Four mismatches in the PAM-distal end have been shown to cause off-target effects. The target loci's sequence homology makes the first type of off-target effects likely to occur, while the second type of off-target effects occurs at off-target locations that are present elsewhere in the genome" **[13].** Sometimes large deletions and genomic rearrangements are caused by off-target effects, which can cause irreversible damage to the recipient and result in fatal genetic changes that impair gene function and, in the end, give rise to cancer cells in animals and undesired phenotypes (disease sensitivity) in plants which also enter the food chain and effect the entire ecosystem and the human element in it.

2.2 The risk of Mosaicism

Mosaicism is another serious risk associated with CRISPR. Mosaicism is when a person has 2 or more genetically different sets of cells in his or her body. While in a normal individual, each cell has 46 chromosomes grouped in 23

pairs. A person with mosaicism may have a mix of cells, some with 46 and others with 47 chromosomes, leading to various health problems. **[14]**Genetic mosaicism, which is the presence of more than two alleles on an individual, is frequently noticed after zygotes have been microinjected with the CRISPR gene editing system. It's when a single person has many genotypes. This arises when gene editing fails to edit the DNA of all relevant cells, so the embryo inherits a mixture of edited and unedited cells. This could leave any resulting child vulnerable to the genetic disease that the editing was supposed to prevent. Mosaicism may also complicate genotype analysis in human individuals having chromosomal abnormalities. A mosaic individual may, for example, be diagnosed as being normal (46, XY) whereas in reality, he/she may have a syndrome such as Turner's (45, X) or Klinefelter's (47, XXY) (Youssoufian and Pyeritz, 2002) **[15].**

2.3 Safety concerns

Our planet has a delicately balanced fragile ecosystem. CRISPR put the power of editing the genes of certain species presumable first for the ultimate benefit of mankind forgetting that the humans compose only one small part of the planet's ecosystem. Do human beings have the right to change he ecosystem to serve their own benefits without a clearer understanding of the implications of these change on the rest of the ecosystem and its fragile equilibrium? It was in this context that renowned scientists like biotechnologist Kevin Esvelt, who is counted among the pioneers in developing CRISPR and proposing its use for gene drives, warned of the ecological risk of releasing these systems into the wild, as it would be tantamount to creating a new invasive species. This ability of trying to manage ecosystems by altering wild populations to suit the interests of human beings could have profound implications for our relationship with the rest of the natural ecosystem and risking something that science does not have accurate and complete knowledge of. For example Biofuel generation using genome-edited microorganisms relies on the alterations (Hemalatha et al., 2023) **[16].** Released modified bacteria must be monitored for interactions with natural species and the large-scale deployment amu have ecological repercussions.

The risks and the safety concerns have multiple dimensions that need to be carefully accounted for before allowing CRISPR to become a common tool. The first safety and risk concern is related to the problem of offset targets and mosaicism discussed above, the second is associated with the fall out of disturbing the delicate balance of the ecosystem and our environment in relation to the use of genome editing on animals, plants and microbes. The third is related to its misuse by non-state players and governments using it for the creation of harmful agents relevant in the bioweapons context; having long term implications for international security.

The COVID 19 pandemic could be a very useful learning example in understanding this safety context. It also showed us how unprepared the world was in the face of the debilitating virus. CRISPR has the potential to create even more dangerous gene edited pathogens that could be leashed on vulnerable population as a weapon of war.

CRISPR-Cas9 could become a tool of bioterrorism with the capability to alter pathogens to make them more transmissible or fatal. Alternatively, it could turn a non-pathogen, such as a harmless microbe, into an aggressive virus. It was in this context, that the director of the US National Intelligence, James Clapper, possibly termed gene editing "weapons of mass destruction and proliferation" in 2018. This statement should be taken seriously as In 2018 itself, the US government released its first bio-defence strategy, involving multiple government agencies, clearly acknowledging the threat associated with gene editing. The risk is even graver when we realise that Gene editing technologies such as CRISPR are getting cheaper and easier to work with, which makes it easier for rogue scientists or organizations/ Nations to misuse them **[17].**

Such concerns also revive the fear expressed in the past. Where researchers spoke about risk that arises from the exploitation of international inconsistencies in biosafety and biosecurity about the governance of genome editing experiments. These inconsistencies create an environment where risky experiments might be carried out in countries with no legal framework (European Commission nd) , or in countries where, although legal frameworks exist, their implementation cannot be achieved due to limited resources (Dickmann et al. 2015) **[18].** The PLA's keen interest in CRISPR is reflected in strategic military writings and research is becoming a model for others to emulate. Biology is among seven "new domains of warfare" discussed in a 2017 book by Zhang Shibo, a retired general and former president of the National Defense University, who concludes: "Modern biotechnology development is gradually showing strong signs characteristic of an offensive capability," including the possibility that "specific ethnic genetic attacks" could be employed. The worrisome aspect is that it could be a cheaper way out for non-state terrorist organisations looking to equip themselves to cause damage.

It is time for the world to reflect on the statement of Dr. Pierre Noel at the Atlantic Council panel on gene editing in September 2016. "It's possible that in the future, as the technology becomes more sophisticated, countries may be able to implement gene-editing technology to design…super soldiers…with great muscle force and strength**."[19]**

Colonel Michael Ainscough highlighted the bioweapon threat in his paper for the USAF Air War College where he wrote about non-state terror groups, "are looking for any opportunity to use limited resources to inflict maximum damage to the U.S. Advances in genetic engineering have offered them an opportunity to create a low cost, low profile, potentially catastrophic weapon of mass destruction; a bioweapon". "Biotechnology has made it possible to inflict mass casualties using only small-scale special operations that can evade detection in attempt to avoid retribution. In asymmetric warfare, biological weapons are seen as a 'great equalizer." he added.

2.4 Impact on Future Generations

The ability to alter our biological makeup, while offering groundbreaking opportunities for healthcare, poses a severe challenge of placing too much power in the hands of medical practitioners in terms of how this technology could impact future generations.

CRISPR-Cas9, the 'genetic scissors', creates new potential for curing diseases; but treatments must be reliable. In a new study, researchers have discovered that the method can give rise to unforeseen changes in DNA that can be inherited by the next generation. These scientists therefore urge caution and meticulous validation before using CRISPR-Cas9 for medical purposes **[20].**

The concern that editing cells could accidentally make changes to sperm or egg cells that can be passed on to future generations without proper accountability and its long-term repercussions is a fraught with risk. "We have a long way to go," Dr.Farahany said. "Until we can figure out what the off-target effects are, and how we can control for them," embryo editing of any kind "would be deeply unethical." **[21].**

2.5 Misuse of technology for personal bias and effort to design and fashion perfect progeny.

The harshest criticism for the Chinese scientist He Jiankui's revelation of the birth of the first babies with edited genes in his laboratory, came from the WHO itself, who weighed in with the most authoritative statement yet on the use of Crispr to alter the DNA of human babies by making a categorical statement asking countries to put a stop to any experiments that would lead to the births of more gene-edited humans. The WHO's director-general put out a statement urging "that regulatory authorities in all countries should not allow any further work in this area until its implications have been properly considered." **[22]** The debate continues. However, there continues to be no international consensus in the matter. While the Human germline editing is already effectively banned in the US, because of a law preventing the US Food and Drug Administration from even reviewing clinical trial applications involving genetically modified human embryos. Russai on the other hand has had a much laxer approach. The fear is that the desire to create perfect babies also termed as designer babies will create a society obsesses with perfection and could destroy the diversity that is the hallmark of our planet.

The development of genome editing technology could bring in pressure that most parents would be unable to withstand due to the fear of their children being left behind given the perfect genetic code of the designer babies. The fear of having children with health problem could be another motivation to take up the offer. "If the resources are available to do so, and other parents are utilizing the technology to create a perfect human, what disadvantages exist for a non-designer individual? Additionally, disadvantages can be realized in any life stage: as a baby, when competing for attention in daycare and subliminal human tendencies result in increased care for the more attractive designer baby; as a child, in an educational setting; and finally, as an adult in the workplace. Under this pressure, an ethical dilemma develops in that a parent's decision to utilize the technology may become less of a reflection on their ethical beliefs, and more on the coercion induced by societal pressure" **[23].**

"Worse, if we use germline gene editing overzealously, it may harm future generations, by removing valuable forms of human diversity" **[24, 25].** There is an ethical context to this argument as there is a growing gear that the rich will be able to afford these designer babies creating a privileged class that would consider everyone inferior. Since genetically designed babies will be healthier and are likely to conform better to societal standards of beauty than non-genetically modified humans, it would create a huge obvious chasm between the haves and the have nots further exacerbating the socio-economic fault lines that exist in societies across the world. It could also lead to a possible widening of the health gap between rich and poor, both within a society and between nations. **[26].** There is the added risk of deepening bias based on colour and ethnicity and physical attributes.

2.6 Misuse of technology for augmenting physical prowess through gene doping.

The sporting arena with billions of dollars and immeasurable personal and national glory attached to it is becoming a domain for genetic engineering or enhancing physical fitness, muscle strength and improving athletic skills of the athletes in various sports.

Doping was banned by the International Olympic Committee (IOC) and the World Anti-Doping Agency (WADA), since the early 1920s and yet it remains a contentious issue. Not there is the growing anxiety around research on gene doping and gene delivery technologies to improve athletic performance in various sports and the lack of means to detect the change.

According to the published data, gene doping is associated with the introduction into the body of the transgene and/or recombinant protein to bring it to expression or to modulate the expression of an existing gene to achieve the further advantage of an athlete's physiological performance **[27, 28, 29].** According to the list of prohibited substances published by WADA in 2008, gene doping has been defined as: "nontherapeutic use of cells, genes, genetic elements, or modulation of gene expression, having the capacity to enhance athletic performance" **[30].** In 2013 WADA clarified the type of manipulation of genetic material prohibited in sport as the transfer of nucleic acids or their analogues into cells and the use of genetically modified cells **[31]**; a move that demonstrated the misgivings about the misuse of this technology for gaining unfair advantage in sports. Sportsmen are looking for gene editing to increase endurance, physical strength, redistribution of fat or increase of muscle mass, control the distribution of oxygen to the tissues, or regulate the growth and/or regeneration of muscle tissue. In addition, gene doping considers the genes encoding the peptides that relieve pain (e.g., endorphins and enkephalins) – they can be used as prohibited analgesics **[32, 33].** Then there is the therapy involving the *EPO* gene which

encodes a glycoprotein hormone that increases the number of red blood cells and the amount of oxygen in the blood, thereby increasing the oxygen supply to the muscles **[34, 35],** which greatly enhances athletic performance.

The endless possibilities of the misuse of gene therapy are keeping the Scientists supported by the WADA on their toes given the difficulty in detection of this intervention. are looking for effective methods and tests for the detection of gene doping used currently in sport.

3. Conclusion

Over the past few years, CRISPR has been making headlines and hailed as a discovery that has the potential to transform the planet. CRISPR is rapidly gaining ground and acceptance from the scientific community as an enormously powerful tool for synthetic biology to generate microorganisms for a broad range of applications, from the production of pharmaceuticals, biofuels, or chemicals to the remediation of pollution or disease diagnostics and treatment. Its relatively precise and easy to use mechanism, its cost effectiveness and scientific impact is making it very popular launching many a startup wanting to encash on this technology.

CRISPR has the shown potential to cure life threatening and disabling genetics health concerns like Hypertrophic cardiomyopathy (HCM) a heart condition that affects roughly 1 in every 500 people worldwide, cancer which is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020 according to a WHO report, diabetes, a disease borne by 422 million people worldwide, the majority living in low-and middle-income countries with 1.5 million deaths reported on account of it **[36]** , various mental ailments like Alzheimer's and many more. Above else, it promises to create babies that do not carry the burden of this heritable disease with a high chance of a reduced or eliminated risk of ever catching it later in life. These eventualities have the capacity to reduce health care burden of nations and revolutionise healthcare with a promise of a disease-free world.

It does not end here. It can revolutionise agriculture by introducing high yielding and nutritious crops to provide for the ever-increasing demand for food across the world that is able to flourish despite the environmental changes that have damaged agriculture across the world**.[37]** Moreover, the mechanism is user friendly and regraded as safer. For example, TALENs and CRISPR-Cas can be used for precise genetic manipulation without introducing exogenous DNA such as antibiotic-resistant genes, thus eliminating the fear that foreign DNA may be present in the final product **[38]**; a fear that has plagued the GMO crops for long.

CRISPR could soon be used to make livestock healthier to meet the growing demand for farm products and meat. CRISPR in farm animals targets a gene called myostatin, which codes for a protein that controls muscle growth. This could be used effectively to have healthier and more muscular farm animals resulting in more emit. Using CRISPR to target the myostatin gene, scientists in Japan have generated red sea bream that are bigger and heavier, with 17% more muscle than their unmodified counterparts. **[39]** Its use in producing more biofuel and the endless possibilities of its use to make the environment safer include bringing back extinct animals like elephant-mammoth hybrid that could keep global warming in check **[40].** The list is endless.

The question that is troubling the global community revolved around the ethics of CRISPR. For example, who takes responsibility for the unintended consequences of changing the genetic code in human embryos? Who takes responsibility for genetically modifying mosquitoes and plants, which might serve the interests of human element of the planet but disturb the ecosystem and its delicate balance in unknown ways.

The world is very polarised in its opinion with a lax China giving scientists a free run to experiment on human embryos while the United States, Canada, or the United Kingdom still to give full clearance. In Canada, editing genes that can be passed down to future generations is a criminal offense with a maximum penalty of ten years in jail. Its application for military purposes is again an issue that generates fear because it's 'easy to use' and 'cost-effective' asset could turn into a curse if it is used for creating biological warfare tools. Moreover, as nations and biotech corporations race against time to make the most of this technology, there is a growing fear that it could spiral out of control if left unregulated and if it were to fall into the hands of unscrupulous elements who could misuse it. Current national and international regulations are highly inadequate with each nation coming up with their own set of rules for regulating and overseeing the usage of this technology **[41].** This lack of a proper regulatory system has also led to a lot of public mistrust in the system, which could thwart scientific advancement and legal uses of CRISPR. Current guidelines, like those put forth by the World Health Organization (WHO) or the National Academies of Science (NAS), are not strong enough and enforceable across international boundaries making them ineffective regulatory measures. In the absence of such measures, CRISPR will continue to be used in an unregulated manner throwing up unwarranted surprises and challenges for the world community just as it did when He Jiankui, the Chinese researcher stunned the world with his announcement of successfully producing genetically edited babies. A well-coordinated centralised international forum with powers to make laws and regulations strictly enforceable internationally to penalise the misuse of this discovery is the only way to keep it safe and prevent it from becoming the proverbial Frankenstein's monster. A cautious approach with a careful understanding of its long-term implications is in the interest of the planet and should not be ignored any longer.

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