

Considerations of Unlimited Applications of Gene Editing

Miyako Takagi

Abstract—CRISPR-Cas9, a current mainstream method for gene editing, has been broadly used by biologists due to its speed, simplicity, and low cost. It allows medical scientists to alter DNA with greater precision than previously existing gene editing techniques. In April 2015, Chinese scientists for the first time edited the DNA of human embryos using CRISPR-Cas9. Although the team had no intention of creating so-called designer baby born from an embryo which has been genetically modified to produce desirable traits, the experiment set off shock waves across the globe. Critics have warned that interfering with human genes could have unintended, negative consequences on future generations.

CRISPR-Cas9 is not a complicated technology, and biohackers have attempted to alter their own genes to promote muscle growth or nullify human immunodeficiency virus (HIV). Furthermore, a scientist posted a “do-it-yourself (DIY) Human CRISPR Guide” online and tried to sell DNA for \$159. Regarding such new DIY bioengineering movements, the regulatory framework has failed to curb these issues not only in the United States but also in many other countries. However, in January 2018, the World Anti-Doping Agency (WADA) officially added genetic engineering to its list of banned substances and methods, with the updated list including “gene editing agents designed to alter genome sequences and/or the transcriptional or epigenetic regulation of gene expression.” Although the use of gene editing for doping has not been reported, the development of evaluation methods that can detect gene editing is warranted before the Tokyo Olympics and Paralympics in 2020.

Index Terms—CRISPR-Cas9, DIY-bio movement, gene editing in sports, human germline editing.

I. INTRODUCTION

Gene editing has enabled scientists to alter an organism’s DNA by adding, deleting, or modifying the DNA at a specific location in the genome. Several approaches to gene editing have been developed. A recent tool known as CRISPR-Cas9, which allows precise editing of genes inside living cells, has been transforming biology. It created a lot of excitement in the scientific community because of being faster, cheaper, more accurate, and more efficient than other existing gene editing methods.

Application of gene editing for the prevention and treatment of human diseases is of great interest to medical researchers. Current research on gene editing has been

focused on understanding the mechanisms underlying diseases using cultured cells and animal models. Scientists are currently elucidating the safety and efficacy of gene editing in the treatment of a wide variety of diseases, including single-gene disorders such as hemophilia and sickle cell disease. Therefore, gene editing holds promise for the treatment and prevention of even complex diseases, such as cancer and HIV infection.

Ethical concerns arise when a gene editing tool as simple and powerful as CRISPR-Cas9 is used to alter the human genome. Most modifications introduced by gene editing are limited to somatic cells and affect only specific tissues. Currently, because of ethical and safety concerns, both germline cell and embryo genome editing are considered illegal in many countries. However, in April 2015, Chinese scientists edited the DNA from a human embryo for the first time [1]. Additionally, in Britain, a group of scientists obtained a license to perform gene editing experiments on human embryos [2].

II. GENE EDITING USING CRISPR-CAS9

In gene editing, the target DNA sequence in a cell is cut at a specific location to inactivate a problematic gene or to insert a replacement DNA sequence for repair for producing the desired result. DNA contains genes and other sequences, whereas a genome refers to the entirety of the hereditary information contained in genes and chromosomes within cells. In humans, a copy of the entire genome (>3 billion DNA base pairs) is contained in each cell that has a nucleus. Currently, the accuracy of gene editing is low, and inaccurate editing may occur; therefore, it is not being viewed as an established technique.

In 2012, molecular biologists Jennifer Doudna at the University of California, Berkeley, and Emmanuelle Charpentier at the Max Planck Institute, Berlin, developed a new technology for gene editing called CRISPR-Cas9 [3]. Subsequently, CRISPR-Cas9 was chosen as the “2015 Breakthrough of the Year” by the US scientific journal “Science.”

CRISPR-Cas9 was rapidly adopted for broad use in biological research due to speed, simplicity, and low cost. This technique allows medical scientists to alter DNA with greater precision than previously available techniques. The CRISPR-Cas9 system consists of two key molecules that introduce an alteration in the DNA sequence. One component is an enzyme called Cas9, which acts as a pair of molecular scissors that can cut the two strands of DNA at a specific location in the genome, thereby adding or deleting a part of DNA. The other component is a small pre-RNA segment

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Miyako Takagi is with the Faculty of Human Welfare, Tokyo Online University, Tokyo, Japan (e-mail: takagi.miyako@internet.ac.jp).

(approximately 20 bp) called guide RNA (gRNA), which is located within a larger RNA scaffold. This scaffold binds DNA, and the gRNA guides Cas9 to the specific location within the genome, ensuring that cutting is performed at the correct location in the genome.

gRNA is designed to locate and bind a specific sequence in the genomic DNA. The bases contained in it are complementary to those of the target DNA sequence. Thus, in theory, gRNA only binds the target sequence and not any other sequence in the genome. Cas9 follows gRNA to the target location and makes a cut across both DNA strands. At this stage, the cell recognizes the damaged DNA and tries to repair it. Thus, the DNA repair machinery can be used to introduce alterations in one or more genes in the genome of a target cell.

III. GENE EDITING IN HUMAN EMBRYOS

A. *Gene Editing in Human Embryos by Chinese Scientists*

Human embryonic DNA was edited for the first time in April 2015 by Chinese scientists. Although the scientists did not intend to create a designer baby, the experiment set off shockwaves across the scientific community. A team of researchers at the Sun Yat-sen University, Guangzhou, injected 86 nonviable embryos with CRISPR-Cas9 to modify the gene responsible for beta thalassemia, a fatal blood disorder. Of the 86 embryos, 71 survived, of which 54 were genetically tested. It was found that only 28 embryonic genomes were successfully spliced and only four contained the modified genetic material. The researchers also observed several off-target mutations caused by the CRISPR-Cas9 system.

Although this research paper triggered a major debate regarding the safety of the procedure, in February 2016, Human Fertilization and Embryology Authority, United Kingdom, approved the application of a research group from the Francis Crick Institute to renew their license for performing gene editing of human embryos.

B. *Ethical Concerns Regarding Human Germline Editing*

To date, more than 40 countries, including 15 in the Western Europe, have discouraged or banned research on germline editing due to ethical and safety concerns [4]. There was also an international effort led by the US, UK, and China to harmonize the regulation on the use of gene editing technologies. This effort was officially launched in December 2015 under the "International Summit on Human Gene Editing" in Washington DC [5].

Due to the possibility of introducing off-target alterations and mosaicism (some cells carry the edited DNA but others do not), safety is of primary concern. At the International Summit on Human Gene Editing, it was widely agreed that germline editing should not be used for clinical reproductive purposes until its safety has been sufficiently proven because the risk cannot be justified by the potential benefit of this method. There is growing concern about gene editing applied not only for therapeutic purposes in humans but also for

nontherapeutic and enhancement purposes, which many scientists view as controversial. This issue should be managed through policy and regulation.

Some scientists have been concerned about obtaining informed consent from prospective parents because it is generally an embryo that undergoes gene editing, and the risks involved in germline therapy remain unknown. However, many believe that research on gene editing in embryos is important for addressing scientific questions about human biology, and that such experiments should be permitted as long as the gene editing products are not used for reproductive purposes. China and UK have already allowed gene editing research on both nonviable and viable embryo leftover following IVF treatments or on embryos specifically created for research. Nevertheless, each study will be dependent on ethical standards of the respective countries.

In Japan, the regulation on using gene editing in gene therapy was initiated in June 2018. As previous clinical research guidelines for gene therapy did not assume gene editing, it was not applicable. Following the new regulation, clinical research on human embryos and germ cells using gene editing has been forbidden [6].

IV. GENE THERAPY USING GENE EDITING

Sangamo Biosciences has developed an HIV treatment that involves isolating immune cells from patients' blood, editing a gene that boosts resistance to the virus, and injecting the cells back into the patient. To date, 80 patients with HIV infection have received the therapy in first-stage trials and have shown positive results [7]. Thus, the technique holds potential to treat genetic disorders such as sickle cell anemia and muscular dystrophy by correcting the responsible DNA sequence. Even if therapeutic gene editing eventually succeeds, its affordability in developing countries remains unclear. Furthermore, even if gene editing becomes a useful strategy for HIV treatment, the cost involved would make the access to such treatments impossible for people living in low-resource countries.

V. GENE EDITING IN SPORTS

A. *Prohibition of Gene Editing in Sports by WADA*

WADA is an international organization created in 1999 to promote, coordinate, and monitor the fight against doping in sport in all its forms. Hoping to preemptively limit the potential consequences of genetic engineering in sports, WADA has officially added genetic engineering to its list of banned substances and methods. The updated list has banned the use of gene editing agents designed to alter genomic sequences and/or the transcriptional or epigenetic regulation of gene expression in January 2018 [8].

B. *Gene Doping Added to the Forbidden List*

Items newly added to WADA Prohibited List January 2018 edition include the following:

[M3] GENE DOPING

The following with the potential to enhance sport performance have been prohibited.

- 1) Use of polymers of nucleic acids or nucleic acid analogs

2) Use of gene editing agents designed to alter genome sequences and/or the transcriptional or epigenetic regulation of gene expression

3) Use of normal or genetically modified cells

WADA has defined gene doping as the nontherapeutic use of cells, genes, or genetic elements or the modulation of gene expression which can enhance performance.

Concrete methods for these three newly prohibited items are shown below:

For 1) Using drugs called “nucleic acid medicines”

For 2) Performing genetic modification and gene editing

For 3) Injecting cells into the body that have been genetically modified or edited outside the body

C. Detection of Gene Editing in Athletes

Gene editing enables small alterations in the DNA of the existing genes or temporarily boosts or switches off the activity of target genes. These alterations can be restricted to specific tissues, such as the muscle, and hence may not be identified in blood tests. Some athletes may even claim that the alterations are not due to gene doping but due to a gene mutation.

Although the use of gene editing for doping has not been reported, the development of evaluation methods that can detect gene editing is warranted before the Tokyo Olympics and Paralympics in 2020.

VI. GENE EDITING BY DIY

A. First Attempt of Gene Editing Using CRISPR-Cas9 by DIY in the US

On October 13, 2017, a former NASA biochemist and currently deemed a biohacker J. Zayner (Ph.D.) became the first person to use CRISPR-Cas9 to alter his own genome [9]. Biohackers are generally scientists, engineers, artists, designers, or activists who experiment with biotechnology outside of conventional institutions and laboratories.

Zayner performed the DIY gene therapy on his left arm while live streaming the procedure on the Internet. Because CRISPR-Cas9 is not a complicated technology, he simply obtained one DNA segment containing Cas9 and gRNA targeted to exon 1 of the myostatin gene. Myostatin inhibits the growth of muscles. He selected the myostatin gene because it has been extensively studied and produces a visible change if gene editing has been successful. After the DNA was injected in his arm, it was expected to enter some of the body cells where the CRISPR-Cas9 system would target the myostatin gene and cut it. If the gene editing procedure is successful, myostatin will not inhibit muscle growth; thus, muscles will show excessive growth. Zayner has provided the world with the means to use CRISPR-Cas9 by posting a DIY Human CRISPR Guide online and selling the required DNA segment for \$159. However, other CRISPR experts believe that the experiment is unlikely to work because the gene is mostly influential during early stages of life, when muscles are being developed.

In another biohacking experiment on October 18, 2017, a computer programmer R. Tristan performed an unproven gene therapy experiment on himself for HIV treatment, which was developed by a biohacking startup Ascendence Biomedical

[10]. In this therapy, he injected gene therapy material into his stomach fat for producing N6, an antibody that is incredibly effective against HIV. He has yet to see any positive results.

B. Regulation for DIY Biomovement

In US, the Food and Drug Administration (FDA) must be notified prior to testing any unapproved drug in humans. Scientists performing such experiments are required to obtain approval; however when the subject is the researcher himself, as in Zayner’s case, approval is not required. This remains a gray area that the FDA does not regulate; hence, Zayner’s experiment can be considered legal [9]. The ethics of obtaining informed consent is relevant even for self-experimentation. Informed consent is the principle that the volunteers in the experiment have fully understood the procedure, are aware of all the risks involved, and have given their consent for participation in the experiment before its initiation. Thus, on November 21, 2017, it was announced that FDA is aware that gene therapy products intended for self-administration and DIY gene therapy kits are available to the public, and that the sale of these products is illegal [11]. In Germany, biohacking is now considered illegal, and individuals attempting such experiments outside a licensed laboratory are liable for punishment of €50,000 fine or 3 years in prison [12].

VII. DISCUSSION AND CONCLUSION

Increasing interest in gene editing as the next generation of technology has been making a serious impact. The latest CRISPR-Cas9 technique is more accurate, more efficient, and cheaper than the existing ones. Human gene editing could have serious consequences depending on the level of reliability and potential applications. Gene therapy in somatic cells is safe to some extent because it does not alter the human genome in perpetuity. However, gene editing has the capacity to impact human heredity for a long time.

Critics have claimed that alterations in human inheritance could have consequences not foreseeable for several generations and would pass genetic alterations to future generations without their consent. An error could potentially have irreversible consequences. There are also concerns regarding babies designed by their parents for better intellect, athleticism, or appearance rather than preventing disease occurrence.

Regarding the potential benefits and risks of this technique, there is a strong need for individuals, including citizens, lawmakers, bureaucrats, scientists, and legal and ethical experts, to perform informed and detailed discussions, so that human gene editing will truly contribute to enhancing the well-being of humans.

Should we restrict the use of gene editing? Many individuals believe that gene editing of embryos should be prohibited. However, human perceptions may change with time. The use of contraceptive pills, in vitro fertilization, and artificial heart was unacceptable decades ago. However, currently, only a few individuals believe that it is a taboo; in fact, many individuals are readily using these methods. In the future, gene editing may become indispensable and may be widely used. Elimination of mutations is of great risk because it is against natural evolution. In the future, humans may find it impossible to respond to the natural environment.

WADA officially added genetic engineering to its list of banned substances and methods. A major problem is that it is difficult to detect gene editing in athletes, and there is no established method for its detection.

In the 1980s, R. Goldman (Founder of American Association of Sports Medicine) conducted a survey on 198 international athletes [13]. The survey question was “If gold medals are guaranteed by doping, even if the probability of death within 5 years is very high, will it be used ?.” More than half of the athletes answered affirmatively. From the first survey until the 1990s, the same survey was conducted every 2 years, and the results remain unchanged. Given the psychology of such athletes, doping using gene editing may be used in the near future. Therefore, it is desirable to establish an inspection method for gene editing in the immediate future.

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Miyako Takagi is a professor in Faculty of Human Welfare of Tokyo Online University. Interest in her research is various ethical issues in medical science. She is a member of the Gene Therapy Review Committee in The Institute of Medical Science, University of Tokyo and also she is immediate past president in Asian Bioethics Association. She received her Docteur d’Université ès Sciences, Université PARIS VII. One of her research, with the title of “Ethical issues in medical and health care innovation” was published on *Eubios Journal of Asian & Int’l Bioethics*, vol.15 No.4 pp.125-126, Nov. 2016.