Why do we need regulatory guidelines for genetic manipulations?

Aditi Jha[1], Mahi Jain[1] and Vijay Rani Rajpal[2]

[1] B. Sc (H) Botany II nd Year Department of Botany, Hansraj College Delhi University, Delhi 110007.

[2] Professor, Department of Botany, Hansraj College Delhi University, Delhi 110007

Abstract

As Science soars past our imagination and decodes life, one begins to wonder what the implications of fiddling with nature could be. Genetics allows us to re-design ourselves, but to what end? Will we live in a world where we imbibe all that is essential and desirable and reject diseases and disabilities? Is it truly possible to choose what we pass on to our future generations and modify the unborn into a superhuman? Will we become a different species altogether or does our conscience warn us against the unpredictable consequences? And what could be the impacts of an easy and democratic access of such genetic manipulation technologies even to bio-hackers or for uses in modifying bacteria or viruses as biological warfare materials? In this article, we explore the potential that the future of gene therapy holds with respect to the above aspects. We will gain a deeper insight into the advancement of genetics and medical technologies and their implications that can affect the quality of life of an individual. Gene editing, a recent and modern domain of Genetics, has been extensively researched and has added immensely to our knowledge of gene functions and their modification. Albeit the discrete regulatory legislations, guidelines, safety and ethics might challenge its growth. Nevertheless, such modern technologies offer us the opportunities to detect and manage many diseases in the unborn and improve their chances of a normal life.

Key Words: Genetic disorders, Gene Editing, Gene Therapy, CRISPR-Cas9, Safety regulations and guidelines

Introduction

Disabilities are an unfortunate event not only for the affected individual but also for their family. They not only challenge the life of the affected individual at the biological front but also ripple out his social identity and limit his capabilities. Substantial research has been conducted to understand and manage many disabilities, disorders and diseases with advancements in medical sciences. Dr. Emmanuelle Charpentier & Dr. Jennifer A. Doudna have offered the world a technology that serves as the benchmark for modern genetic research. They are also the first two women-only awardees of The Nobel Prize in Chemistry on October 7 2020, for their work on CRISPR-Cas9 to edit genes and thereby their functions.

GENES AND GENE EDITING

"Genes" are biological parcels of information passed on from a parent to an unborn and a

common source of amusement to many parents as they wonder how their newborn inherited their nose and eyes. However, the information-packed in genes does not limit its influence to physical similitude; it is in fact the 'biological code' that gets expressed in the child. Sometimes, this code is faulty. Genetic disorders present a grave challenge as their spread is not just restricted to the affected individual but also has the potential to get transmitted on to the next generations. The manifestations of genetic defects can range from minor gene function alterations to lethality. Caused by mutation(s) in one or many genes or damage to chromosomes coupled with environmental influence; these can be classified into: a) Single gene disorders b) Chromosomal disorders and c) Complex disorders.

Modern technologies offer countless possibilities in the form of Gene therapy and Gene editing to fix or correct the above disorders. Gene therapy is the practice of tracking down the culprit gene and replacing it. The contemporary developments in Medical technology under the umbrella of 'Gene Therapy' have been remarkable in saving the affected person and his future generations from a compromised living standard by either preventing or curing the genetic disease permanently.

The temptation to step beyond the restraints of necessity and open doors to numerous miracles has prodded us to alter genes identified with specific tangible traits. This quest offers a chance of enhancing one's capabilities beyond the ordinary potential in the desired direction. By re-structuring genes, one can change their manifestations via a process termed 'Gene Editing'. The main difference between gene therapy and gene editing lies in the objectives that can be achieved via either process. While gene therapy limits its scope to treating prospective or pre-existing genetic diseases, gene editing involves making minor changes in the DNA of organisms to customize their physical traits desirably.

HISTORY OF GENE EDITING

The principles governing gene therapy took roots, back in the 1950s when it was proposed in the double-stranded helical model of the DNA that the arrangement of nucleotide base pairs in a specified way impacted their expression (Watson and Crick, 1953). Extensive experimental research was conducted to study and modify these sequenced base-paired codes for desired effects. As a result, it became possible to alter these codes in bacteria and viruses to produce desired expressions and products (Cohen and Boyer, 1972). These experiments were then conducted in organisms with larger genomes and eventually could be performed in humans after identifying the defective gene, isolating the correct and functional gene attached to a molecular marker by using a restriction enzyme, cloning the functional gene using the Polymerase Chain Reaction and inserting it into the human genome in place of the defective gene (Mullis, 1985).

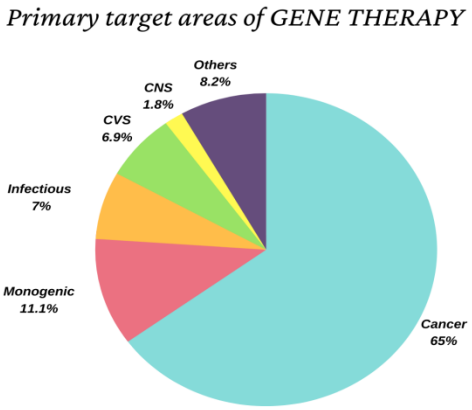
Gene therapy can be broadly categorized as, Somatic and Germ-line Gene therapy. Somatic gene therapy primarily involves the introduction of a correct and functional copy of a gene(s) into the somatic cells of a person suffering from a genetic disorder for instance in the case of patients suffering from Cystic Fibrosis and Adenosine deaminase deficiency. Since somatic cells include all the cells of the body exclusive of the sperm and egg cells the

effects of somatic gene therapy are not heritable (Gluzman, Carter & Muzyczka, 1984).

Germ-line gene therapy, on the contrary, replaces faulty copies of the gene(s) in the sperm and egg cells and hence results in preventing diseases in the off-springs (W.F. Anderson,1990). While somatic gene therapy effectively helps the diseased individual, it is however a comparatively tedious process given the number of somatic cells needing the corrected gene. Furthermore, genetically modified DNA would not be passed on to subsequent generations. Germ-line gene therapy, on the other hand, bears the potential to eliminate the disease from the entirety of successive generations and operates at a much primitive stage in the organism's life hence facilitating a healthy early development and not damage control later on.

Upon compilation of the reports on the usage of gene therapy, it was found that Cancer is the primary target of most clinical gene therapy trials and forms 65% of all the trials (Samantha L Ginn, 2017). Many types of cancers have been targeted using gene therapy either by the insertion of tumour-suppressing genes or by the usage of oncolytic virotherapy. Next in line are Inherited Monogenic diseases constituting nearly 11% of all the clinical gene therapy trials wherein insertion and/or transfer of just one functioning gene to the dividing stem cells can guarantee a permanent correction. Other disorders like the ones associated with the Cardiovascular and the Central Nervous System come in next and together constitute nearly 9% of all the clinical gene therapy trials, they are targeted mainly at degenerative neurological diseases like Alzheimer's and Parkinson's.

Germ-line gene therapy and any form of heritable genetic modification cannot be practised in India as per the guidelines issued by the Indian Council of Medical research with inputs from the Central Drugs Standard Control Organization and the Department of Biotechnology- released in July 2019. The guidelines made suggestions related to future legislation on gene editing.



TOOLS FOR GENE EDITING

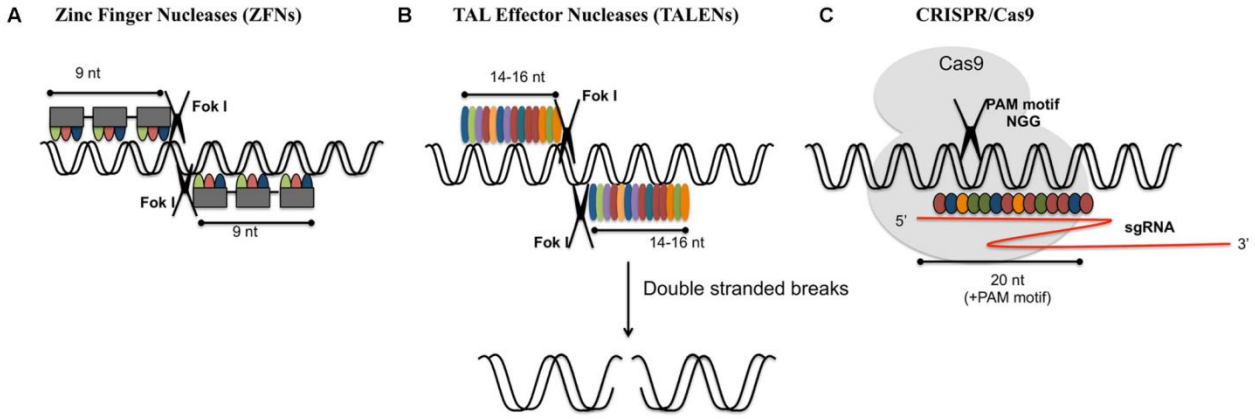
The magnitude of changes caused by these techniques cannot be fathomed without developing a clearer insight into how they operate. The three most important underlying mechanisms include- Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), Transcription Activator- Like Effector Nucleases (TALEN) and Zinc-Finger Nucleases (ZFN).

1. Zinc-finger Nucleases are artificially designed restriction enzymes, they are made up of DNA Binding and DNA Cleavage Domains which help bind the ZFN to the gene

which is identified as a target, they then cleave the gene at the particular site. DNA binding domains of ZFNs are generally found to have 3 to 6 ZF repeats recognizing 3X base pairs. The specificity of ZFNs is due to the binding motifs as they are designed based on the sites they target (Aaron Klug, 2017). *Fok*I is a cleavage domain belonging to the Type II class of restriction endonucleases. It forms a dimer with and cleaves the DNA. ZFNs can be designed to target desired DNA sequences. ZFNs have been used to edit the genomes of Tobacco, Sea Urchin, *Arabidopsis*, Silkworm, Soybean and various mammalian cells (D. Carroll, 2011).

2. Transcription Activator-Like Effector Nucleases (TALENs) were discovered in *Xanthomonas* (Boch et al, 2009). They are injected into plant cells through Type III secretion systems and bind specifically to DNA that is based on a 34 amino acid repeat motif. It does not recognize the promoter sequences of genes that enable bacterial infection. They can be fused to form functional domains and applied to edit the genome. The DNA binding motifs are engineered based on the coding amino acids in the TAL motif. The DNA sequence at the restriction site of the restriction enzyme *Fok*I is fused to the DNA sequence for the TAL repeats causing the creation of a chimeric enzyme that is capable of targeting specific DNA regions.

3. Cas-9 also known as CRISPR-associated protein 9, was earlier known as Cas5, Csn1 or Csx12. With a mass of 160 Kd, it is a protein that plays an important immunological defence role in certain bacteria protecting them against invading viruses (DNA) & plasmids. It is extensively used in applications of genetic engineering. The enzyme cleaves specific targeted sequences of DNA complementary to the CRISPR sequences which it uses as a guide (PD Hsu, 2014). CRISPR-Cas System is a nuclease system guided by RNA; it introduces specific cleavages in the dsDNA and is found to be functional in all commonly found model organisms. To cleave a target site in a genome, one needs an endonuclease and a homing device (RNA acting as guide/ Cas nuclease/ gRNA). The Cas 9 nuclease is then directed to search for the complementary sequence and generate a double-stranded break (PD Hsu, 2014). This mechanism, which bacteria have developed over mega-annums to protect



(Credits: Arora Leena & Narula Alka (2017) Gene editing and crop improvement using CRISPR-cas9 system. Frontiers in Plant Science. 8. 1932. 10.3389/fpls.2017.01932.)

themselves from invading viruses, is now available as a simple, cost-effective, time-saving and easily available alternative to researchers and scientists for editing any DNA segment or gene. Thousands of experiments have been conducted globally with CRISPR based gene editing and many groundbreaking discoveries have been made using it in the past decade.

A modestly equipped lab with trained technicians can use CRISPR to edit and modify bacterial/viral/ human genome. This development, however, is both exhilarating and a concerning alarm.

FUTURE OF GENE EDITING

The noble aim of medical technologies such as the ones discussed is to improve the quality of our life. DNA editing technologies would pave the way for scientists to correct mutations that lead to genetic disorders, diseases and disabilities While they can be seen to do just that in the case of genetically afflicted patients, it becomes ethically questionable when alterations are made in order to lighten the skin-tone, darken the hair and babies are designed according to the characteristics desired by the parents or what is acceptable to a particular society or culture. While there are no barriers to the endless pursuit of our whims and fancies, there are certainly harms that can potentially arise out of a situation such as this one. Genetic enhancement in such cases can be anticipated to amplify the personal and cultural biases that already exist within our communities. It reduces genetic diversity- something that is crucial for our existence. It incentivizes companies to effectuate a desirable workforce and nations to design stronger armies. It takes away individual identities shaped by values passed on and experiences incurred in one's lifetime- it in essence, takes away whatever makes a human, *human*. The situation becomes really precarious when one considers the deployment of gene editing for creating deadlier pathogens by modifying bacterial/viral genomes to create biological warfare materials to make more infectious, more transmissible pathogens to hit global economies without a political and/or economic fallout and to plan bio-conspiracies and execute biohacking. All these in addition to mounting turmoil and deadlier consequences can be foreseen to arise if technologies like Gene editing are not tightly regulated universally.

With regard to Gene Editing, one of the first reports arrived in the year 2014, wherein a successful genetic modification was shown to be accomplished in monkeys at an early developmental stage (Yuyu Niu, 2014). In 2015, an obscure and largely unknown journal published the learnings of a Chinese team stating the usage of CRISPR on human embryos for the first time. However, the success rate was only 7.4% with a total of four embryos surviving with intended changes from a pool of 54 embryos. In the two years that followed, the technology took multiple strides and the success rate was currently shown to be nearly 4 times higher than in 2015, from the six embryos used for study three showed the desirable changes. In April of the year 2016, a similar group of Chinese Scientists reported the modification of human embryos to make them HIV infection resistant. In the instance of prenatal gene alteration in the Chinese twins- Lulu and Nana, done with the intention of making the twins immune to HIV was claimed to impact

their cognitive functions bound to provide them with a natural advantage over others. (He Jiankui, 2019). This act was largely deemed irresponsible and legal action was called for.

The most prodigious stride, however, was seen in late 2018 and early 2019 when scientists and researchers changed their approach from the classical technique of "breaking the double-stranded DNA" and letting it repair itself which results in errors to "base editing" which go on to swap nucleotides for the desirable ones and the success rate thus, increased to 89% (Junjiu Huang, 2015). The biggest disclosure came about in the year 2018 when a Chinese group (He Jiankui, 2018) claimed to design 'First CRISPR Human Baby" whose genome had been edited before birth. This became a seed for global outrage and Jiankui was put behind bars. There was a tremendous uproar that invited considerable public engagement and started inquiring into the ethical aspects of editing genes. Lately, heated debates and concerns have been raised against the misemployment of genetic manipulation techniques like CRISPR to create deadly synthetic viruses and pathogens for usage in biological warfare (J Van Aken, 2003) demonstrating the darker aspects of the misapplication of disciplines such as Science and technology. Over the last six months, humankind has witnessed so many controversies surrounding the origin of the Novel Covid-19 virus amidst speculations of gene editing.

AFFIRMATIVE ACTION

The line between ethical and unethical gene-editing practices is obscure. To prevent its misuse, well-defined policies should have been declared or treaties established to strictly define the 'limits' to the utilisation of CRISPR based gene editing in animals and human beings. There should have been Governmental policies with a clear cut understanding of the restrictions of areas wherein editing of genes is not allowed. Unfortunately, before all these regulations and frameworks were put in place, the technology was already widespread globally.

In a damage control exercise, scientists from seven countries around the world have called upon for a five-year moratorium on all clinical uses of human germline gene editing to make genetically modified children until the guidelines are drafted by an independent regulatory authority which governs the creation, amendment and safe execution of gene editing (Eric S. Lander, 2019). Such a body will need to list the limitations and restrictions to the purpose of gene editing. Laws deterring violation must also be set in place as a means to curb illegal practices.

It is clear that Science and modern technologies are developed targeting the well-being of humans. But the irony of the situation claims that the same technologies are at the discretion of crooked and dishonest Scientists and Bio-hackers as well. To prevent their misuse, we should give time for Gene editing technology to mature, international regulatory guidelines for its use to get established to thoroughly assess the safety measures, to gauge regulatory clarity and the limits on the categories of use.

Acknowledgements and Declarations

We thank Dr. Rama, Principal Hansraj College for providing us with this opportunity and for

her continuous support. The ideation was made possible on account of the knowledge received from Late Mr. Priyansh Khandelwal on consent and ethics. We extend our heartfelt gratitude to Dr. Vijay Rani Rajpal for her patience and guidance at all times.

REFERENCES

- Journal Article by Arora L, Narula A. (2017) Gene Editing and Crop Improvement Using CRISPR-Cas9 System. Department of Biotechnology, School of Chemical and Life Sciences, Jamia Hamdard University, New Delhi, India *Front Plant Sci.* 8;8:1932. doi: 10.3389/fpls.2017.01932. PMID: 29167680; PMCID: PMC5682324.
- Journal Article by Ayanoğlu, Fatma Betül et al. (2020) "Bioethical issues in genome editing by CRISPR-Cas9 technology." *Turkish journal of biology = Turk biyoloji dergisi* vol. 44,2 110-120., doi:10.3906/biy-1912-52
- Journal Article by Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, Lahaye T, Nickstadt A, Bonas U. (2009) Breaking the code of DNA binding specificity of TAL-type III effectors. Department of Genetics, Institute of Biology, Martin-Luther-University Halle-Wittenberg, Weinbergweg 10, D-06099 Halle (Saale) Germany. *Science.*11;326(5959):1509-12. doi: 10.1126/science.1178811. PMID: 19933107.
- Article by Carroll D. (2011) Genome engineering with zinc-finger nucleases. Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, Utah 84112-5650, USA. *Genetics*.;188(4):773-82. doi: 10.1534/genetics.111.131433. PMID: 21828278; PMCID: PMC3176093.
- Article by Cohen, S N et al. (2011) "Construction of biologically functional bacterial plasmids in vitro." Center for the Study of Science and Innovation Policy. *Proceedings of the National Academy of Sciences of the United States of America* vol. 70,11: 3240-4. doi:10.1073/pnas.70.11.3240
- Journal Article by Coller, Barry. (2019). Ethics of Human Genome Editing. Allen and Frances Adler Laboratory of Blood and Vascular Biology, Rockefeller University, New York, NY 10065, USA, *Annual Review of Medicine.* 70. 289-305. 10.1146/annurev-med-112717-094629.
- Article by Ginn SL, Amaya AK, Alexander IE, Edelstein M, Abedi MR. (2019) Gene therapy clinical trials worldwide to 2017: An update. *J Gene Med*. 2018 May;20(5):e3015.
- Journal Article by Gyngell, Christopher et al. (2017) "The Ethics of Germline Gene Editing." *Journal of applied philosophy* vol. 34,4: 498-513. doi:10.1111/japp.12249
- Encyclopedia Chapter by Hill W G, (2001) "Selective Breeding" in *Encyclopedia of Genetics*
- Article by Hsu, Patrick D et al. "Development and applications of CRISPR-Cas9 for genome engineering." Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, MA 02141, USA *Cell* vol. 157,6 (2014): 1262-1278. doi:10.1016/j.cell.2014.05.010
- Review Article by Joung JK, Sander JD. (2013) TALENs: a widely applicable technology for targeted genome editing.

Nat Rev Mol Cell Biol.;14(1):49-55. doi: 10.1038/nrm3486. PMID: 23169466; PMCID: PMC3547402.

- Article by Klug A. (2010) The discovery of zinc fingers and their development for practical applications in gene regulation and genome manipulation. MRC Laboratory of Molecular Biology, Cambridge CB2 0QH, UK.Q *Rev Biophys*. doi: 10.1017/S0033583510000089. PMID: 20478078.
- Journal Article by Lander ES, Baylis F, Zhang F, Charpentier E, Berg P, Bourgain C, Friedrich B, Joung JK, Li J, Liu D, Naldini L, Nie JB, Qiu R, Schoene-Seifert B, Shao F, Terry S, Wei W, Winnacker EL. (2019) Adopt a moratorium on heritable genome editing. *Nature*;567(7747):165-168. doi: 10.1038/d41586-019-00726-5. PMID: 30867611.
- Research Paper by Marsden, G. & Towse, A. & Pearson, S.D & Dreitlein, B. & Henshall, C. (2017) "Gene Therapy: Understanding the Science, Assessing the Evidence, and Paying for Value,"
- Journal Article by Miquel-Clopés, A et al. (2019) "Mucosal vaccines and technology." *Clinical and experimental immunology* vol. 196,2: 205-214. doi:10.1111/cei.13285
- Journal Article by Niu Y, Shen B, Cui Y, Chen Y, Wang J, Wang L, Kang Y, Zhao X, Si W, Li W, Xiang AP, Zhou J, Guo X, Bi Y, Si C, Hu B, Dong G, Wang H, Zhou Z, Li T, Tan T, Pu X, Wang F, Ji S, Zhou Q, Huang X, Ji W, Sha J. (2014) Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. *Cell.* 13;156(4):836-43. doi: 10.1016/j.cell.2014.01.027PMID: 24486104.
- Article by Srinivas R K et al, (2016) "Asian Biotechnology and development review", Vol. 18 No. 1 ISSN: 0972-7566
- Journal Article by Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD. (2010) Genome editing with engineered zinc finger nucleases. *Nat Rev Genet*.;11(9):636-46. doi: 10.1038/nrg2842. PMID: 20717154.
- Journal Article by Watson JD, Crick FH. (1953) Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. *Nature.*;171(4356):737-8. doi: 10.1038/171737a0. PMID: 13054692.
- Journal Article by Weichselbaum RR, Hallahan DE, Sukhatme VP, Kufe DW. (1992) Gene therapy targeted by ionizing radiation. *Int J Radiat Oncol Biol Phys*.;24(3):565-7. doi: 10.1016/0360-3016(92)91075-x. PMID: 1399745.