GSJ: Volume 8, Issue 2, February 2020, Online: ISSN 2320-9186
www.globalscientificjournal.com

GENOME EDITING USING CRISPR AND ITS ETHICAL ISSUES

Nejat Siraj Mohammed[1]*

* Ankara University, Institute of Biotechnology, nejusiraj@gmail.com

ABSTRACT

ClusteredRegularlyInterspaced Short Palindromic Repeats (CRISPR) are shortmotifs of DNAcommonlyfound in chromosomal and plasmid DNA, which are separated with other uniquesequences.Eventhough it was discovered in a bacteria, it has been applied in healthcare, environmentalbiology, veterinaryscience and agriculturaltrialswith themost popular applicationbeing in cancerresearch.The technique is commonly used on somatic cells for gene editing; However, its use in germline cells stillethicallycontroversial. The main ethicalissues of gene editing by CRISPR that are discussed in this review are; safetyissues, its misuse, unequalaccess to the classes of the population and ecologicalconsiderations. Some unsolvedtechnicalcomplications in CRISPR and limitedknowledge of human germ cells are some of the obstacles for performing a safe gene editing in humans. In addition to this, due to uncontrolledusage and limitedaccess, the technology may serve for the individualpurposes of some people. Futureperspectivesincludingregulatedusage as well as equalaccess are also other points of concern which would be achieved by introducing a consensus among all countries.

Keywords*: CRISPR, gene editing, germ cells, ethics, regulations.*

1. INTRODUCTİON

1.1 Gene Editing

In November 2018, a Chineseresearchgroupheadedby He Jiankui of the SouthernUniversity of Science and Technology in Chinainformed that theysuccessfullymodifiedembryos to be HIV tolerant by deleting their CCR5 gene using CRISPR technology. They also claimed that some of theseembryoswerelater used to createpregnancies that led to the birth of two HIV toleranttwins (which are nowdubbed the CRISPR twins). The news was faced with different feedbacks from scientists, governmentsectors as well as the public. While some supported the research, most, however, had expressed their fears and concernsaboutmisuses and ethicalissues that should be regardedabout this so-called CRISPR technology.

The process of gene editing is not a newapproach; in fact,scientistswere in search of ways to editgenes since the 1960s. The discovery of Restriction Enzymes in the late 1960s and early 1970's and its application as recombinanttechnology, has tremendouslyincreasedtrialstargeting genomic changes in different types of cells(1).Thus, the first targeted genomic changeswere performed in yeast and mice cells. In these first gene editingtrials, homologousrecombination techniques werecommonly used. This was surprisinglyprecise but very inefficient, particularly in mouse cells(2).Nowadays gene editing has been madeeasy due to the discovery ofcertain techniquesincluding CRISPR-Cas9, ZFNs or TALENs, viral systemslike rAAV and transposons(3).

1.2 CRISPR System

ClusteredRegularlyInterspaced Short Palindromic Repeats (CRISPR) are shortmotifs of DNA commonlyfound in chromosomes and plasmid, which are separated with other uniquesequences. (4). CRISPR was first discovered in 1987in the *Escherichiacoli*genome as an aggregateof repeatedbits of DNA containing 29 nucleotideswhich are separated bysequences of variablesizes(5).

The CRISPR locus, involved in acquiredimmunitysystems arepresent in about90 % of archaea and 45% of bacteria(6).Thesemicroorganisms use the CRISPR-Cassystemto destroy the genetic material of the virus or another genetic element that attacksthem(7). CRISPR-Cassystemscontain RNA-guidednucleases that are currently considered to be the mosteffectiveappliancesof genomeediting and engineering(5).One of the best-

investigatedCRISPR systems is the CRISPR IIsystemthatcontainsthe nuclease protein known as Cas9. The CRISPR-Cas9 systemstarts its action by incorporatingforeign DNA into the CRISPR array. This is followed by the recognition of foreign DNA using CRISPR components. Next, the spacersequences are transcribed into a CRISPR RNA (crRNA) that can be a guide RNA that can bind with Cas9 protein which in turncuts the target DNA(8). The same procedure is followed in the laboratory with the onlydifference being the artificial synthesis of the guide RNA. The main function of this RNA is to bind with the target DNA and at the same time with the Cas9 enzyme that cuts the DNA.Eventhoughthere are many enzymesrelated to the CRISPR system, Cas 9 is the mostcommonly used one. Once the DNA is cut, the required genetic material is inserted to the cut or deleted from it. This procedure is performed by using the cells own DNA repairmechanism(9).

1.3Application of CRISPR MediatedGene Editing

CRISPR technique has greatlycontributed to the scientificworld as it provides a cheap, effective and precise gene editing technique. It is been applied in healthcare,environmentalbiology, veterinaryscience and agriculturaltrialsthat involveddifferent experimentalmodels, includinghumans(10). However; the mostcelebratedapplication of this technique is incancerresearch. So far, CRISPR has been used to identify and findtreatments for various types of cancers. For instance, at least 15 clinicaltrialson different types of cancers and other diseases have beenintroducedwith CRISPR applications(11). CRISPR/Cas9directed gene editing can be practicedon humangenes in three different ways.These are; to obtain knowledge on the processof development of human cells or embryos to developnewtreatments. Secondly, it can be usedfor genetic editing in somatic cells, to tackle various illnesses. The third and mostethicallycontroversial one isits usage in germlineediting to rule outmutations in the next generation(12).

Scientistsdon’tdeny the roleof CRISPR in the treatment of various genetic disorders in humans.However, theydon’t use it to its highest extent due to the ethicalconcerns that arise with it.Thus the main aim of this review is to explorecertainethicalargumentsregarding gene editing of germ line cells, gametes, and embryos using CRISPR techniques.

2. THE ETHİCS OF CRISPR TECHNOLOGY APPLİCATİONS

Different people have different viewsabout the use of this technique in gene editing. A study carried out by Blendon, Gorski&Bendon in 2016 revealed that the community'sviewsregarding the technique change when used in different aspects. Itturned out to be that the communitysupports it when it is used in adults but opposes it when used in embryos. The scientificcommunity, on the other hand, had broadviews on the topic. Scientists indicated that the technique is "acceptable when its benefits, both to individuals and the broadersociety, exceed its risks, ... though the relevantrisks and benefits and levels of acceptable risk are todayuncertain" and on the other handshowedconcerns on the unpredictablefuturerisks and its control that may outweigh the benefits(13).

Moral decisions in Bioengineering is one of the important aspects of the field. To consider the efficacy of a technique one has to investigatethe strongsides, the outcomes and the sideeffects it can give. Oncethesepoints are fullyunderstood, it would be appropriate for humans to use it. The same process goes for CRISPR. Generally, the main ethicalconsiderations and debatesabout CRISPR technologyfall into main fourpoints.These are safetyconcerns, its misuse or uncontrolled use, unequalaccessibility, and ecologicalconcerns.

2.1 SafetyIssuesRegarding Gene Editing by CRISPR

The main safetyconcerns of using the technology for gene editing are based on the defects of the technology and ourlimitedknowledge of humangerm line cells. Much has been knownabout CRISPR technology since its discovery. However, many problems and concernsregarding the technique must be addressedbefore its application on humangermlines One of the main hindrancepoints for the technology is targetingdifficulties. Non-HomologousEndJoinrepairing (NHEJ) is one of the mechanisms used by cells to repairbreaks in the DNA. Many CRISPR techniques use this method as a way of achieving the highest targetingaccuracytowards the gene of interest. However,these techniques may result in deletions and insertions which wouldresult inseriouschangesto the genes.It has been known that the same technicalerrors have occurred in the abovementionedChinese CRISPR twins study which resulted in different baseinsertion and deletions(14). Another serioustechnical problem that has to be addressed is off-targetmutations. This kind of mutation has been shown to increase when using the CRISPR/Cas9 system. Thesemutations can result in genomic instability anddisruption of normal genes(15). Although

thesetechnicalissuescreateloopholeswhile using the technique, it isbelieved that theycould be resolvedover time with increasedexperimentation and trials.

In addition to this,anotherreason to be concernedabout the safety of the technique arises due to ourlimitedknowledgeabout the human germ cells. This is to say that right now the longtermconsequences ofgermline genetic modification is unclear and mightposerisks to the individual(16). Again; a typicalexample of this case is the Chinese CRISPR twins;Lulu andNana. According to some scientists, the twinsmight have a greatercognitive ability due to the genetic alteration on the gene CR55.Alcino J. Silva, a neurobiologist at the University of California, Los Angeles, confirms this fact. He explains that the modifications in gene CR55 will probablyaffect their brains' cognitive ability. He also stated that due to the unpredictableoutcomes, such kinds of trialsshould not be performed(17).

2.2 IncreasedMisuse of Technology

The secondethical issue that couldarise is the probability ofits misuse in the future. The technology that is designed for therapeuticreasonsmightendup to be used to fulfillindividualdesires.There is a fear that uncontrolled use of the technologymightlead to some form of eugenics which favorsbreeding ofonly the “enticing”breed of the humanrace(11). Aside from therapeutical, people may use the technology to edit the genes to have preferabletraits like longposture and blueeyes. In myopinion, nontherapeutictrials can undergostrictguidelinesjust for the sake of research. But the main concern is that some among us mightdare to tryselectivemodifications for the population at large, therebypossiblyirreversiblyaltering the humanspecies(18).In this case, strictlaw and regulationemphasizing the application of the technologyto different purposes must be drafted and takenseriously. But it must be notedthat banningCRISPR based genome researches or any other researchcannotbringlong-lastingsolutions to ethicalconcerns.Besides, many countries of the World have alreadyestablishedguidelinesabout researches on the humanembryo.The government of the United States of America, for example, does not provide funds to supporthumanembryotests(14).On the other hand, scientists in China have a betteropportunity to experiment with gene editing on humanembryos(19). This inconsistencyof the laws in different countries mustbe avoided to regulatetrials. Besides this,strict controls over privatelyfunded researches must also be part of the regulation for gene editing.

2.3 Unequal Access to the Technology

The secondethicalconcern that arises with this technology is its equalaccessibility by the different economicclasses of the community. There is a fear that once gene editing by this technique is made legal, individuals can grow apart from each other and establishclasses based on the quality of their engineered genome(20).The argument in this pointstarts with the fact that CRISPR occurs in naturalbacteria and that it is not an inventedtechnology. If the technologyhappens to be the property of a researcher,most of the lower economicclassindividuals may not getaccess to use the technique even for therapeuticreasons. This may result in the existence of various numbers of communityclustersdifferentiated from each other based on their genomes and their access to the CRISPR based healthcaresystem(21). In addition to this, the differingaccess to technology can open the door to some sort of eugenics. As FrançoiseBaylismentioned in an interview with the Journal of ClinicalChemistry in 2016, the risks of racism, sexism, and others may be increased as an aftermathof different levels of accessibility to the technique (18).Besides,some countries may use this CRISPR based genome editing (aimed at enhancing the IQ, physicalendurance…etc.) to gainsignificantmilitary and economicpowers(22).

2.4 The EffectsCRISPR on Ecosystems and Biodiversity

Through the action of CRISPR/cas9 in a procedurecalled gene drive (a condition that encompassesinheritingcertain "selfish"geneallowing a mutation or foreign gene to spread quicklythrough a populationwherethey can causedeath or infertility); scientists have already indicated that it is possible to eradicatemosquitoesonce and for all(23)(24). Specific species of mosquitoes are the main vectors for deadlyhumandiseases like Malaria and Zika virus infection. Well, would it be a good idea to eradicatemosquitoes to stop thesediseases? While the eradication of Mosquito from the environment may not seem to affect life on earth, from an ecologicalpoint of view, it can result in irreversibledamage to the ecologicalbalance.While such kind of geneticallymodifiedorganisms can be used in the lab for research,care must be taken for them not to join the ecosystemwithoutundergoing an extensiveinvestigation of the ecologicalchangestheymightbring. KevinEsvelt, an ecologist at MIT oncesaid "Wego from the defaultassumption that the thingsweengineer will not spread, to assumingthey will. Normallyyou can make any kind of fruitfliesyouwant—naturalselection will wipe the floor with them. But as soon as you'rethinking of a gene drivetechnology, you have to

assumewhateveryou'remaking will spread once it getsoutside the lab. Human error will win out, if notdeliberatehumanaction."(25)

Many plants or animals have extincted from the surface of the earth due to climatechanges or due to various actions of humanbeings. De-extinctionrefers to the action of bringingbackspecies thatcannot be found on earthanymore(26). Through the usage of the CRISPR technique, many laboratories are trying to recreate some of the long-goneanimals. BobbyDhadwar, fromChurch'slab, is one of the leadingresearchers in the field and intends to bringback the extinctwoollymammoth. Dhadwarplans to do this by insertingsinglenucleotidepolymorphisms (SNPs) of a woollymammoth into immortalizedAsianelephant cells. After this process, they will use CRISPR /cas9 to combine all SNP insertions into one cell.They would insert this into an Asianelephantembryo, which would eventuallycreatewoollymammoth(27). Twomainconcernsarise here: first, as it has been alreadystated that ourknowledgeregarding the use of the technique is still very limited. In this case; a technique gonewrong can create a disastrousoutcome. Hence many if not all, parts of the genome are manipulated, it mightcreateunbearablesuffering to the deextinctedanimal. The second issue is the spread of the genes of the animal into nature and their control later.This might not be a problem for the woollymammoth but is a point that has to be consideredbefore applying it to smallerextinctanimals.

3. CONCLUSİON

CRISPR can be considered one of the most important discoveries of the centuryas it has enabled humans to explore the pathogenesis and therapeuticalternatives for many diseases and mutations. Eventhough the technique is commonly used on somatic cells for gene editing; its use in germline cells stillethicallycontroversial. The main ethicalissues of gene editing by CRISPR that are discussed in this review are; safetyissues, its misuse, equalaccess to the classes of the population and ecologicalconsiderations. The effect of off-target andtargetingdifficultiesare considered to be the main technicalfaults that can be seen in CRISPR. Thesepoints added with limitedknowledge of germ cells to the presentdaymakes the use of CRISPR in germ lines one of the hot ethicalissues in biotechnology.Futureperspectivesincludingregulatedusage as well as equalaccess are also other points of concernwhich would be achieved by introducing a consensus among all countries.

DECLARATİON

EthicsApproval and Consent to participation

Not applicable.

Consent for Publication

Not applicable.

Availability of Data and Materials

Not applicable.

Competing of Interests

The authorsdeclare that they have nocompetinginterests.

Funding

Not applicable.

AuthorsContribution

NS have collected literatures, assesedthem and drafted the review.

Aknowledgement

I would like to thankAssistantprofessor Dr. Yeşim Doğan from Ankara University for supporting me and controllingmymanuscript.

4. REFERENCES

1. Bleicher A. Genome Editing Before CRISPR: A Brief history. 2018.
2. Kelly E. Ormond DPM, Derek T. Scholes, Yvonne Bombard, Lawrence C. Brody, W. Andrew Faucett, Nanibaa' A. Garrison, Laura Hercher, Rosario Isasi, Anna Middleton, Kiran Musunuru, Daniel Shriner, Alice Virani, and CEY. Human Germline Genome Editing. Am J Hum Genet. 2017;101(2):167–76.
3. Gene editing [Internet]. 2019. Available from: https://www.horizondiscovery.com/gene-editing

4. Viviane Richter. What is CRISPR and what does it mean for genetics? [Internet]. Available from: https://cosmosmagazine.com/biology/what-crispr-and-what-does-it-mean-genetics

5. Ishino Y, Krupovic M, Forterre P. History of CRISPR-Cas from Encounter with a Mysterious Repeated Sequence to Genome Editing Technology. Margolin W, editor. J Bacteriol [Internet]. 2018 Jan 22;200(7). Available from: http://jb.asm.org/lookup/doi/10.1128/JB.00580-17

6. Bakr Shabbir MA, Hao H, Shabbir MZ, Hussain HI, Iqbal Z, Ahmed S, et al. Survival and evolution of CRISPR-Cas system in prokaryotes and its applications. Front Immunol. 2016;7(SEP):1–14.

7. DRLAARM L. The CRISPR-Cas immune system: Biology, mechanisms, and applications. Biochimie. 117:119–28.

8. Questions and answers about CRISPR. 2019; Available from https://www.broadinstitute.org/what-broad/areas-focus/project-spotlight/questions-and-answers-about-crispr

9. Medicine UN library of. What are genome editing and CRISPR-Cas9? 2019.

10. Rodríguez-Rodríguez DR, Ramírez-Solís R, Garza-Elizondo MA, Garza-Rodríguez MDL, Barrera-Saldaña HA. Genome editing: A perspective on the application of CRISPR/Cas9 to study human diseases (Review). Int J Mol Med. 2019;43(4):1559–74.

11. Brokowski C, Adli M. CRISPR Ethics: Moral Considerations for Applications of a Powerful Tool. J Mol Biol [Internet]. 2019;431(1):88–101. Available from: https://doi.org/10.1016/j.jmb.2018.05.044

12. Rossant J. Gene editing in human development: ethical concerns and practical applications. Co Biol. 2018;

13. Guertin L, Mcguire R, Torres A, Stapleton P. Public Perception of Human Applications of CRISPR Gene Editing An Interactive Qualifying Project to be submitted to the faculty of Worcester Polytechnic Institute in partial fulfilment of the requirements for the Degree of Bachelor of Science. 2018; Available from: https://www.wpi.edu/project-based-

14. Ma Y, Zhang L, Qin C. The first genetically gene-edited babies: It's "irresponsible and

too early." Anim Model Exp Med. 2019;2(1):1–4.

15. Zhang XH, Tee LY, Wang XG, Huang QS, Yang SH. Off-target effects in CRISPR/Cas9-mediated genome engineering. Mol Ther - Nucleic Acids. 2015;4(11):e264.

16. Redman M, King A, Watson C, King D. What is CRISPR/Cas9? Arch Dis Child Educ Pract Ed. 2016;101(4):213–5.

17. Regalado A. China's CRISPR twins might have had their brains inadvertently enhanced [Internet]. [cited 2019 Jun 20]. Available from: https://www.technologyreview.com/s/612997/the-crispr-twins-had-their-brains-altered/

18. Vasiliou SK, Diamandis EP, Church GM, Greely HT, Baylis F, Thompson C, et al. CRISPR-Cas9 system: Opportunities and concerns. Clin Chem. 2016;62(10):1304–11.

19. Victor Tangermann. A CRISPR Future: Five Ways Gene Editing Will Transform Our WorldNo Title [Internet]. Available from: https://futurism.com/crispr-genetic-engineering-change-world

20. Institute NHGR. What are the Ethical Concerns of Genome Editing? [Internet]. Available from: https://www.genome.gov/about-genomics/policy-issues/Genome-Editing/ethical-concerns

21. Mulvihill JJ, Capps B, Joly Y, Lysaght T, Zwart HAE, Chadwick R. Ethical issues of CRISPR technology and gene editing through the lens of solidarity. Br Med Bull. 2017;122(1):17–29.

22. Rath J. Safety and Security Risks of CRISPR/Cas9. 2017;107–13.

23. Hegg J. Is Intentional Extinction Ever the Right Thing? [Internet]. [cited 2019 Jun 20]. Available from: https://blogs.plos.org/ecology/2016/07/01/is-intentional-extinction-ever-the-right-thing/

24. Callaway E. Controversial CRISPR 'gene drives' tested in mammals for the first time. Nature International Journal of Science [Internet]. 2018; Available from: https://www.nature.com/articles/d41586-018-05665-1

25. Adle J. Kill All the Mosquitoes?! SMITHSONIAN MAGAZINE [Internet]. 2016; Available from: https://www.smithsonianmag.com/innovation/kill-all-mosquitos-

180959069/

26. Rogers K. Deextinction. In: Britannica [Internet]. Available from: https://www.britannica.com/science/de-extinction

27. WRAY B. CRISPR May Prove Useful in De-Extinction Efforts. The Scientist [Internet]. Available from: https://www.the-scientist.com/reading-frames/crispr-may-prove-useful-in-de-extinction-efforts-30992