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# A New Vista in Genome-Editing: Are There Concerns

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**ABSTRACT:** *The concept of genome-editing has revolutionised the field of Basic Biology research, medicine and biotechnology. The technologies, through DNA-binding protein, are engineered to cause modification of specific genes. In the process, carry out the needed repair of the selected mutant genes. As a result, it could offer treatment modalities to some diseases afflicting humans. However, the safety profile of the technology only guaranteed with the somatic cells. Recently, a new editing tool clustered regularly interspaced short palindromic repeat (CRISPR)-associated system (Cas) now available. The fact that CRISPR-Cas uses RNA molecules with some degree of human DNA sequence specificity has brought about an attempt at extending its use in the human preimplantation embryo. This consideration has generated serious concern bore out of the poor knowledge of the mechanism of DNA repair in the human preimplantation embryo. As a result, the possibility of off-target, mosaicism could portend catastrophic effect on the future generation.*

**Keywords: Genome- editing, CRISPR, DNA sequence, pre-implantation embryo**.

## INTRODUCTION

The knowledge of the genetic basis of diseases has to lead the revolution of the concept of manipulation of genes for therapeutic purposes initiated with the introduction of gene therapy (Cox *et al*., 2015). Gene therapy is simply the replacement of the disease gene with exogenous good ones. Though progress made over the years, riddled with several challenges. Ranging from the ease of injecting the gene into the cell with the appropriate vector to the subsequent gene expression and its impact on the neighbouring genes which in most cases leads to unintended effects (Kay, 2011). Also, only a few numbers of disease conditions are addressed as most of the dominants gene mutation could not be handled (Cox *et al*., 2015). To circumvent some of these challenges, the concept of genome-editing was developed aimed at modifying the genome sequence for therapeutic purposes.



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**Mechanism of Action**

The device based on the background knowledge of the natural method of cellular DNA repair following double-strand break (DSB). It involves the use of Homologous Direct Repair (HDR) through the use of homologous sequence as a template to initiate repair of the broken end. While the second method is the non-homologous end joining (NHEJ) which simply ligate the broken end (Lieber, 2010). While this approach seems more reliable, it is associated with mutagenesis and prone to deletion/insertion at the point of breaks. In genome-editing, these methods are explored through manipulations that can be either gene deletion with the application of NHEJ or the stimulation of the HDR with the supply of exogenous donor template. HDR is naturally active during the S and G2 phase of the cell cycle where the template generated from the sister chromatid for the repair (Cicia and Elldge, 2010). Genome-editing tends to mimic this physiological process by introducing exogenous template with the vector and the nuclease. The incorporation of the template to the endogenous sequence at the locus brings about the correction of the mutation (Takashi, 2014).

**Repair Strategy;**

The strategy is initiated with the use of Nuclease to induce target DSB. These nucleases are Zinc Finger Nuclease (ZNF), Transcription activator-like effector Nuclease (TALENS), Meganuclease, and CRISPR/Cas9 (Segal and Meckler 2013). Unlike the other Nuclease, the CRISPR/Cas9 which was recently designed involves the use of guide RNA to match any sequence without the need for repeated engineering of proteins which is labour intensive and not cost effective (McNutt, 2015). Also, it allows several changes made at the same time. However, requires the presence of Protospacer-Adjacent Motif (PAM) at its target site. (Makarova *et al*., 2014). The hallmark of genome-editing is to ensure specificity of the nuclease at the target sites. As a result, several efforts have been directed towards this in a bid to advance the technology. Despite this progress, the problem of off-target has remained a major challenge especially with regard complex genome (Cong *et al*., 2013). The success achieved in its application in Agricultural Settings and human somatic cells have engendered interest towards its use in human pre-implantation embryo for therapeutic purposes (Liang *et al*., 2015). However, the concern over its safety and ethical considerations have put it under serious debate among researchers.

**Prospects;**

Genome-editing technologies equipped with several benefits in areas of research and therapeutics. The therapeutic values span through various disease conditions such as birth defects often resulting from genetic disorders to extensive more debilitating diseases such as cancers, Diabetes, and even HIV. It will help to reduce the disease burden significantly among millions of people in the world. Also, it may serve as a better alternative to pre-implantation genetic diagnosis (PGD) and provide a better method of embryo selection. Unlike PGD, it does not require a large number of the embryo to be generated to carry out the necessary intervention (Savulescu, 2012 and McNutt, 2015). Also, its wide coverage involves the correction of multi-complex genetic disorders and as well as carrier



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cases which could pose a risk for the next generation if PGD used in the absence of other healthy embryos (Savulescu *et al*., 2016).

**Challenges;**

**Off-Target;**

One of the primary concern of this technology is the associated off-target which can pose serious harm. The phenomenon has created a significant puzzle in the minds of most researchers because it becomes rather difficult to determine the mutation caused by the technology from spontaneous mutation. Though recent study with mice, tried to diffuse this assertion by the findings of a significant reduction in the off-target mutation with CRISPR/Cas9 genome editing (Maeder *et al*., 2016). On the contrary, the study with human embryo revealed significantly high off-target mutation (Cyranoski and Reardon, 2015). The reason for the sharp contrast was due to the homogenous strains of mice used and the effect of the technology on the variation in genetic status cannot be ascertained (Liang *et al*., 2015). However, to allay the anxiety over the off-target mutation, Iyer *et al*. (2015) have shown that the use of adequate control of the duration of the Nuclease and avoidance of Plasmid base delivery could subvert or reducing the incidence of off-target mutation. In light of this, it was advocated that the research given a chance as no technology can be put into full clinical use unless it is confidently stated to be safe in line with the global concept (Harriton, 2013).

**Mosaicism;**

Another concern is the incidence of mosaicism which stems from the fact that human embryo is very dynamic due to its fast dividing cells. Unlike the somatic cells, the mechanism of DNA repair in the embryo is not well known. However, the application of the technology has tried to explore the use of HDR platform. The limitation associated with HDR is the influence of genome modification such as the size of the sequence and the spate of activity (Cox *et al*., 2015). Thus, resulting in the failure to complete splitting or the cell may end division prior completion of editing consequently leads to mosaicism (Mathew and Lovell-Badge, 2015). The findings were corroborated by Liang *et al*. (2015) in the study with three pronuclear to evaluate the efficacy of CRISPR/Cas9. Most of the DNA repair following DSB were through the error-prone NHEJ, and only 25% of the embryos got fixed through the endogenous template with some degree of competition by homologous genes predispose to unintended mutation. Only 14.3% used the HDR and were all mosaic. Thus suggestive of poor understanding of the mechanism of DNA repair in embryo necessitating more research.

**Possibility for non- therapeutic purposes;**

The non-therapeutic use has generated lots of ethical questions among researchers primarily in settings with weak or in- adequate regulatory framework. The quest for money or pressure from couple may result in the concept of the designer baby (Cyranoski and Reardon, 2015). The consequence of such act might be quite devastating to the future generation. So, there is the need



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for the proper regulatory program in place and as well public enlightenment to avoid baby becoming like a manufactured products.

## CONCLUSION

Genome-editing is a welcome development considering its broad scope of potentials and possible benefits in handling diseases. These perceived benefits must weigh against the risks. Also, the adequate regulatory framework must be put in place for optimal development through extensive research to attain the desired goal.

## REFERENCES

Cox DBT, Platt RJ, and Zhang F (2015). Therapeutic genome editing: Prospects and Challenges. Nature Medicine: Volume 21 Number 2.

Cong L, Ran FA, Cox D (2013). Multiplex Genome Engineering using CRISPR/Cas9 Systems Science, 339: 819-823.

Cicia A and Elledge SJ (2010). The DNA damage response: making it safe to play with knives. Molecular Cell Biology 40: 179-204.

Cyranoski D and Reardon S (2015). Chinese scientists genetically modify in human embryos; Nature News online htt:/www.nature.com/news/Chinese-scientists-genetically-mod.

Harriton M (2013). Genome editing takes off. Lab Animal: 42 No. 10 pp. 343.

Iyer V, Shen B, Zhang W, Hodgkins A, Keane T, Huang X, Skarmes WC (2015). Risk of off-target mutation are rare in Cas9-mediated mice. Nature Methods vol. 12 no. 6: 479.

Kay M A (2011). State of the art gene base therapies: the road ahead. Nat. Rev. Genet. 12, 316-328.

Lanphier E and Urnov F (2015). Don't edit the human germ line, Nature Vol. 519.

Ledbetter DH (2009). Chaos in the embryos, Nature Medicine, 15. Pp 490-491.

Lieber MR (2010). The Mechanism of Double strand DNA Break by the Nonhomologous DNA END joining pathway. Biochemistry, 79:181-211.

Liang P, Xu Y, Zhang X, et al (2015). CRISPR/Cas9- mediated gene editing in human trinuclear zygotes. Protein Cell. 6(5): 363-372.

Maeder ML and Gersbach CA (2016). Genome-editing Technologies for Gene and Cell Therapy; Journal of the American Society of Gene and Cell Therapy: vol. 24 no. 3, 430-446.

McNutt M (2015). Breakthrough to genome editing. Science. Vol. 350 Issue;6267 pp1445.

Mathews DJH, Lovell-Badge R (2015). Human embryos are prime targets for genome editing. A path through the thicket: Nature Medicine Volume 527.

Makarova Ck, Charpentier ES, Koonnin EV (2014). Classification and evolution the types of CRISPR/Cas9 systems. Nucleic Acids Res 42: 6091-6105.

Perez PP, Ousterout DG, Gersbach CA (2012). Advances in targeted genome editing, current opinion: Chemical Biology, 16: 268-277.

Savulescu J, Pugh J, Douglas T, Gyngell C (2015). The moral imperative to continue gene editing research on human embryos: Protein Cell; 6(7): 476-479.

Segal DJ, Meckler JF (2013). Genome engineering the dawn of the golden age; Human Genetics, 14: 135-58.



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Takashi Y (2014). Targeted genome editing. Develop. Growth. Differ: 56. 1.
Tsai SQ, Iafrate AJ, Joyng JK (2014). Towards a functional understanding of variants using genome editing. Nature Medicine, 20: 1103-1104.


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