The first genetically gene-edited babies: It's “irresponsible and too early”

Yuanwu Ma1 | Lianfeng Zhang2 | Chuan Qin1,3

1Key Laboratory of Human Disease Comparative Medicine, National Health Commission of China (NHC), Institute of Laboratory Animal Science, Peking Union Medicine College, Chinese Academy of Medical Sciences, Beijing, China
2Beijing Engineering Research Center for Experimental Animal Models of Human Critical Diseases, Institute of Laboratory Animal Science, Peking Union Medicine College, Chinese Academy of Medical Sciences, Beijing, China
3Beijing Key Laboratory for Animal Models of Emerging and Reemerging Infectious Diseases, Beijing, China

Correspondence
Chuan Qin, Key Laboratory of Human Disease Comparative Medicine, National Health Commission of China (NHC), Institute of Laboratory Animal Science, Peking Union Medicine College, Chinese Academy of Medical Sciences, Beijing, China.
Email: qinchuan@pumc.edu.cn
and
Yuanwu Ma, Key Laboratory of Human Disease Comparative Medicine, National Health Commission of China (NHC), Institute of Laboratory Animal Science, Peking Union Medicine College, Chinese Academy of Medical Sciences, Beijing, China.
Email: mayuanwu@cnlas.org

Abstract
A scientist, Jiankui He of Southern University of Science and Technology of China, recently claimed at the Second International Summit on Human Genome Editing in Hong Kong on 29 November that he has created the world’s first genetically altered babies using CRISPR. This announcement sparked controversy and criticism. The newly developed CRISPR/Cas9 technique has been applied to genetic modification of many kinds of animals. However, the technique is still in its infancy and many questions remain to be answered before it can be used for clinical purposes, especially for reproductive purposes.

Keywords
Animal Models, animal welfare and ethics, Molecular Biology

On 29 November 2018, at the Second International Summit on Human Genome Editing in Hong Kong, the scientist Jiankui He, of Southern University of Science and Technology of China, claimed he has created the world’s first genetically altered babies. This announcement sparked controversy and criticism and was almost universally denounced.
The Chinese Academy of Medical Sciences responded: "we are opposed to any clinical operation of human embryo genome editing for reproductive purposes in violation of laws, regulations, and ethical norms in the absence of full scientific evaluation". The National Health Commission of China responded: "This illegal behavior will be verified and punished". The genetic alteration of human eggs, sperm, and embryos is prohibited for germ line purposes. The relevant guidelines already exist in China. Jiankui He's work violated those guidelines.

CRISPR/Cas9 techniques have been applied in many kinds of animals, including human cells. It is very clear that this system can be used to genetically modify the human germ line today. However, many questions remain to be answered before this technique can be used to alter the human genome for reproductive purposes. Although the intent may be to create perfect human beings, the result may be a monster.

**WHAT IS CRISPR/CAS9?**

Mammalian genomes contain billions of base pairs and are difficult to manipulate. With the development of homologous recombination (HR), we can precisely modify the genome, with expected outcomes. However, precise HR-mediated alteration occurs at a very low frequency (one in $10^9$–$10^7$ cells). A series of programmable nuclease-based genome editing tools, such as Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and the RNA-guided DNA endonuclease Cas9 (CRISPR/Cas9), have been developed in recent years, which enable efficient genetic modifications of many species. The ZFNs were derived from eukaryotic transcription factors, TALENs were derived from *Xanthomonas* bacteria, and CRISPR/Cas9 was derived from the type II CRISPR system. Of the current genome editing tools, the RNA-guided Cas9 system has been developed most rapidly. This system can easily be used to target a genomic locus with a small guide RNA (sgRNA) complementary to the target DNA sequence.

CRISPRs were first reported in *Escherichia coli* in 1987 and are present in over 40% of sequenced bacteria and 90% of sequenced archaea. Currently, the type II CRISPR system, first identified as part of an adaptive immunity system that protects the hosts against invasion by plasmids and other DNA contaminants, is the most commonly used.

Since the first report of CRISPR/Cas9 techniques being used for gene targeting in mammalian cells in 2013, these techniques have been applied in many species. In theory, they can be used for human germ line modification, but there are still many open questions to be solved before any attempts to apply it should be made.

**Targeting difficulties**

All programmable nuclease-based editing tools work via introduction of a site-specific DNA double strand break (DSB). The DSB will stimulate DNA repair through nonhomologous end-joining (NHEJ) and/or homologous recombination (HR)-directed repair mechanisms. HR-mediated repair occurs only in specific phases of the cell cycle (G2 and S), while NHEJ-mediated repair occurs throughout the cell's life. NHEJ-mediated repair is the primary damage-mediated repair mechanism. NHEJ-mediated repair is not an entirely accurate process and may induce small deletions or insertions at the target sequence. In order to achieve very precise genome modification, various kinds of CRISPR-based genome editing tools were developed including adenine base editors (ABEs), cytosine base editors (BE3), and so on. The Cas9 system may continue to work beyond one cell division and may induce small deletions or insertions result in: (a) frameshifts causing a stop codon occurrence at or after DSB sites, which results in elimination of the target gene; (b) frameshifts which introduce a new amino acid strand or protein, resulting in a different protein; (c) deletion of several amino acids. In the second situation, the newly produced amino acid or protein may be toxic and have unexpected consequences. More basic research is necessary to evaluate the safety and validity of these techniques. According to present information, the system which Jiankui He used have resulted in different base insertion and deletions. Using this technique could lead to unintended results for the organism.

**Off-target effects**

The most important concern a newly developed gene editing tool must address before any kind of application is attempted is to show that there are no off-target effects. Based on present data, the CRISPR/Cas9 system does induce off-target mutations. And further, these mutations can be transmitted to the organism's descendants. While we know a lot about this technique, there are still many unknowns. Further research and development of this technology may uncover more unintended off-target and other effects, as well as other unexpected consequences. Such off-target mutations or other effects could lead to cancer or other diseases in the early or later life of genetically modified babies.

**Mosaic issue**

The CRISPR/Cas9 system may continue to work beyond one-cell fertilized eggs and result in a mosaic genotype. This means that different tissues or organs will have different genetic modifications, even within the same organism. We are still uncertain what the effects of the gene editing would be in the genome of babies.

**WHICH IS THE PERFECT TARGET GENE?**

In order to select the perfect target gene and an efficient target site, we need to understand that gene's function well, and the target sgRNA should have very few or no off-target effects. This requires a significant accumulation of knowledge, which is so far lacking. Currently, most knowledge about gene function comes from basic research, which often uses mice missing a gene of interest (called knockout mice) to understand the effects of the gene. However, whether genes function in the same way in mice and humans is still
unclear, and gene function studies in humans are still in their infancy. There is currently very little known about how gene knock-out in humans will affect a person’s behavior, health, and lifespan and how it could be transmitted to their descendants. There is no effective method to evaluate those effects in human beings.

Jiankui He selected the Ccr5 gene as the target gene, with the stated purpose of preventing HIV infection. However, is this the perfect target for HIV prevention? The C-C chemokine receptor 5 (CCR5) is a seven-transmembrane G protein-coupled receptor (GPCR) and is highly expressed in bone marrow-derived cells including T cells and macrophages.32 In other tissues, CCR5 is expressed on epithelium, endothelium, vascular smooth muscle, and fibroblasts.33-35 Many studies have demonstrated that CCR5 has an important role in HIV virus infection.36,37 CCR5 is therefore a potential target for HIV infection protection. In another study, however, Ccr5 gene deletion also showed lupus nephritis susceptibility.38 In the central nervous system, Ccr5 is expressed on neurons, astrocytes, and microglia and functions as a suppressor for cortical plasticity and hippocampal learning and memory.35 The Ccr5 gene function in many tissues is still unclear. Deletion of this gene may result in unexpected disease.39 It is therefore a very risky target for gene editing.

ETHICAL PROBLEMS

The scientific community has already developed a broad social consensus about the application of these techniques. It strongly encourages basic research and manipulation in laboratories, but does not condone use of the technique for genetically altering human babies. We agree with the major recommendations:

Research: “Intensive” research is encouraged and should proceed, including in human germ line cells, subject to appropriate legal and ethical oversight.

Clinical use (somatic): Treating adults with gene editing therapies should proceed within existing regulatory frameworks and guidelines.

Clinical use (germ line): Gene editing for human reproductive purposes is in principle prohibited until all safety and ethical issues can be addressed.

Ongoing forum: The international community should establish "norms" and every country makes its own laws for human gene editing.

Many countries already have principles and guidelines regulating human embryo experiments. In the United States of America, use of federal funds to finance genetic modification experiments in gametes and embryos is prohibited. In China there are already guidelines for genetic manipulation for human reproductive purposes. The guidelines including: Guiding Principles of Ethics for Human Embryonic Stem Cell Research (2003), Ethics Principles for Human Assisted Reproductive Technology and Human Sperm Bank (2003), Ethical Review Measures for Biomedical Research Involving Human Beings (2016), and Safety Management Measures for Biotechnology Research and Development (2017).5

While regulations and guidelines already exist and regulate government-funded studies, but there are few restrictions for privately funded research. The CRISPR technique is still in the initial stages of evaluation and it is premature to consider it for clinical use, especially for reproductive purposes. In order to avoid the birth of “a second CRISPR baby,” we strongly recommend that the government should regulate clinical experiments using this technique for human reproductive purpose.

ACKNOWLEDGEMENTS

The present work was supported in part by National Natural Science Foundation of China (31501001, 81571222), and CAMS Innovation Fund for Medical Sciences (CIFMS) (2017-I2M-3-015, 2017-I2M-2-005).

CONFLICT OF INTEREST

None.

ORCID

Yuanwu Ma https://orcid.org/0000-0002-1882-1777

REFERENCES

1. Wang C, Zhai X, Zhang X, et al. Gene-edited babies: Chinese Academy of Medical Sciences’ response and action. Lancet. 2018; pii: S0140-6736(18)33080-0. https://doi.org/10.1016/S0140-6736(18)33080-0. [Epub ahead of print]
2. SinaTech. The China NHC response of the first gene-edited babies in China. SinaTech Nov 26, 2018. http://news.sina.com.cn/o/2018-11-26/doc-ihpevhck8401332.shtml
3. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome editing. Cell. 2014;157:1262-1278.
4. Urnov FD, Miller JC, Lee YL, et al. Highly efficient endogenous human gene correction using designed zinc-finger nucleases. Nature. 2005;435:646-651.
5. Miller JC, Tan S, Qiao G, et al. A TALE nuclease architecture for efficient genome editing. Nat Biotechnol. 2011;29:143-148.
6. Boch J, Scholze H, Schornack S, et al. Breaking the code of DNA binding specificity of TAL-type III effectors. Science. 2009;326:1509-1512.
7. Moscou MJ, Bogdanove AJ. A simple cipher governs DNA recognition by TAL effectors. Science. 2009;326:1501.
8. Cong L, Ran FA, Cox D, et al. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013;339:818-823.
9. Mali P, Yang L, Esvelt KM, et al. RNA-guided human genome engineering via Cas9. Science. 2013;339:823-826.
10. Wang H, Yang H, Shivalila CS, et al. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell. 2013;153:910-918
11. Ma Y, Zhang L, Huang X. Genome modification by CRISPR/Cas9. FEBS J. 2014;281:5186-5193.
12. Mojica FJ, Diez-Villasenor C, Garcia-Martínez J, Soria E. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. J Mol Evol. 2005;60:174-182.
13. Mojica FJ, Diez-Villasenor C, Soria E. Juez G. Biological significance of a family of regularly spaced repeats in the genomes of Archaea, Bacteria and mitochondria. Mol Microbiol. 2000;36:244-246.
14. Makarova KS, Haft DH, Barrangou R, et al. Evolution and classification of the CRISPR-Cas systems. Nat Rev Microbiol. 2011;9:467-477.
15. Makarova KS, Aravind L, Wolf YI, Koonin EV. Unification of Cas protein families and a simple scenario for the origin and evolution of CRISPR-Cas systems. Biol Direct. 2011;6:38.

16. Li W, Teng F, Li T, Zhou Q. Simultaneous generation and germline transmission of multiple gene mutations in rat using CRISPR-Cas systems. Nat Biotechnol. 2013;31:684-686.

17. Li D, Qiu Z, Shao Y, et al. Heritable gene targeting in the mouse and rat using a CRISPR-Cas system. Nat Biotechnol. 2013;31:681-683.

18. Kim H, Kim JS. A guide to genome engineering with programmable nucleases. Nat Rev Genet. 2014;15:321-334.

19. Gaudelli NM, Komor AC, Rees HA, et al. Programmable base editing of A to T or G to C in genomic DNA without DNA cleavage. Nature. 2017;551:464-471.

20. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016;533:420-424.

21. Shen B, Zhang W, Zhang J, et al. Efficient genome modification by CRISPR-Cas9 nickase with minimal off-target effects. Nat Methods. 2014;11:399-402.

22. Ran FA, Hsu PD, Lin CY, et al. Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. Cell. 2013;154:1380-1389.

23. Mali P, Aach J, Stranges PB, et al. CAS9 transcriptional activators for directed gene expression. Elife. 2013;2:683.

24. Wang X, Wang Y, Wu X, et al. Unbiased detection of off-target cleavage by CRISPR-Cas9 and TALENs using integrase-defective lentiviral vectors. Nat Biotechnol. 2015;33:175-178.

25. Lin Y, Cradick TJ, Brown MT, et al. CRISPR/Cas9 systems have off-target activity with insertions or deletions between target DNA and guide RNA sequences. Nucleic Acids Res. 2014;42:7473-7485.

26. Kuscu C, Arslan S, Singh R, Thorpe J, Adli M. Genome-wide analysis reveals characteristics of off-target sites bound by the Cas9 endonuclease. Nat Biotechnol. 2014;32:677-683.

27. Duan J, Lu G, Xie Z, et al. Genome-wide identification of CRISPR/Cas9 off-targets in human genome. Cell Res. 2014;24:1009-1012.

28. Fu Y, Foden JA, Khayter C, et al. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. Nat Biotechnol. 2013;31:822-826.

29. Ma Y, Shen B, Zhang X, et al. Heritable multiplex genetic engineering in rats using CRISPR/Cas9. PLoS One. 2014;9:e89413.

30. Yang H, Wang H, Shivalilla CS, Cheng AW, Shi L, Jaenisch R. One-step generation of mice carrying reporter and conditional alleles by CRISPR/Cas-mediated genome engineering. Cell. 2013;154:1370-1379.

31. Ma Y, Zhang X, Shen B, et al. Generating rats with conditional alleles using CRISPR/Cas9. Cell Res. 2014;24:122-125.

32. Sorec S, Myburgh R, Krause KH. The chemokine receptor CCR5 in the central nervous system. Prog Neurobiol. 2011;93:297-311.

33. Cartier L, Hartley O, Dubois-Dauphin M, Krause KH. Chemokine receptors in the central nervous system: role in brain inflammation and neurodegenerative diseases. Brain Res Brain Res Rev. 2005;48:16-42.

34. Paruch S, Heinis M, Lemay J, et al. CCR5 signaling through phospholipase D involves p44/42 MAP-kinases and promotes HIV-1 LTR-directed gene expression. FASEB J. 2007;21:4038-4046.

35. Zhou M, Greenhill S, Huang S, et al. CCR5 is a suppressor for cortical plasticity and hippocampal learning and memory. Elife. 2016;5:pii:e20985. https://doi.org/10.7554/eLife.20985

36. Liang M, Kamata M, Chen KN, Pariente N, An DS, Chen IS. Inhibition of HIV-1 infection by a unique short hairpin RNA to chemokine receptor 5 delivered into macrophages through hematopoietic progenitor cell transduction. J Gene Med. 2010;12:255-265.

37. Hunt JS, Romaneli F, Maraviroc, a CCR5 coreceptor antagonist that blocks entry of human immunodeficiency virus type 1. Pharmacotherapy. 2009;29:295-304.

38. Cheng FJ, Zhou XJ, Zhao YF, Zhao MH, Zhang H. Chemokine receptor 5 (CCR5) delta 32 polymorphism in lupus nephritis: a large case-control study and meta-analysis. Autoimmunity. 2014;47:383-388.