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EDITORIAL

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

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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".[1] The National Health Commission of China responded: "This illegal behavior will be verified and punished".[2] 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 10^6-10^9 cells).[3] A series of programmable nuclease-based genome editing tools, such as Zinc finger nucleases (ZFNs),[4] transcription activator-like effector nucleases (TALENs)[5-7] and the RNA-guided DNA endonuclease Cas9 (CRISPR/Cas9),[8-10] have been developed in recent years, which enable efficient genetic modifications of many species. The ZFNs were derived from eukaryotic transcription factors,[4] TALENs were derived from *Xanthomonas* bacteria,[5-7] and CRISPR/Cas9 was derived from the type II CRISPR system.[8-10] 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.[11,12]

CRISPRs were first reported in *Escherichia coli* in 1987 and are present in over 40% of sequenced bacteria and 90% of sequenced archaea.[13] 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.[14,15]

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.[8,10,16,17] 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).[4-10,18] 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 progress 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 editors3 (BE3), and so on.[19-23] NHEJ-mediated 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.[24-29] 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.[10,29-31] 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 knockout 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.[38] 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).[1]

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.

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CONFLICT OF INTEREST

None.

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