Considerations on genetic engineering: regarding the birth of twins subjected to gene edition

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Abstract

In this essay, the bioethical implications of the recent genetic manipulation in human embryos with CRISPR-Cas9 to eliminate the CCR5 gene and the birth of a pair of discordant twin girls are analyzed. The experiment was disseminated via social media. The main bioethical flaws identified include the justification of the model, the informed consent process and the lack of disclosure of evident conflicts of interest. The consequences of the experiment on the life of the twins that were born were not properly evaluated, such as the impact on their autonomy, the alleged benefits to be received and the future risks of harm during their lifetime. Having manipulated the germ cell line, the effects on their future offspring were not considered. This type of actions negatively affects the way society conceives science. Genetic engineering should be reserved to the basic experimental context or as clinical research for the correction of known serious diseases of genetic origin under strict regulatory and bioethical supervision and using a gradualist approach in accordance with the advances of gene editing techniques.

KEY WORDS: Gene editing. CRISPR-Cas9. Bioethics.

Reflexiones sobre la ingeniería genética: a propósito del nacimiento de gemelas sometidas a edición génica

Resumen

En este ensayo se analizan las implicaciones bioéticas de la reciente manipulación genética en embriones humanos con CRISPR-Cas9 para eliminar el gen CCR5 y el nacimiento de dos gemelas discordantes. El experimento se divulgó en medios sociales. Los principales problemas bioéticos identificados son la justificación del modelo, el proceso de consentimiento informado y la falta de declaración de evidentes conflictos de interés. No se evaluaron apropiadamente las consecuencias del experimento sobre la vida de las gemelas nacidas como la afectación a su autonomía, los supuestos beneficios por recibir y los riesgos futuros de daño durante su vida. Habiendo manipulado la línea celular germinal, no se consideraron los efectos sobre su descendencia futura. Este tipo de acciones tiene un impacto negativo en la forma como la sociedad concibe la

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ciencia. La ingeniería genética debe reservarse al contexto experimental básico o bien como investigación clínica para la corrección de enfermedades conocidas graves de origen genético, bajo estricta supervisión regulatoria y bioética y de manera gradualista de acuerdo con el progreso de las técnicas de edición genética.

PALABRAS CLAVE: Edición genética. CRISPR-Cas9. Bioética.

Introduction

By the end of November 2018, a story was released stating that a pair of twin girls that had been born a few weeks prior had undergone genetic engineering (genetic surgery, according to the promoter) by applying the CRISPR-Cas9 technique, when they were at embryonic stage, allegedly to protect them against the human immunodeficiency virus (HIV), which was carried by their father.

In Mexico, at the College of Bioethics we have established a reflective and analytical discussion about the particular case and, more broadly, about genetic engineering in the biomedical field. In this manuscript, we present the main arguments and reflections resulting from said discussion.

Human beings have learned to manipulate the genetic characteristics of living beings since thousands of years ago, even without knowing how and why such changes occur. Agriculture and domestication of some species of non-human animals are clear examples (*teocintle* is little recognizable as the plant that originated current corn). Scientific knowledge has allowed understanding the mechanisms whereby these evolutionary phenomena occur.

Genetic engineering brings together various tools and techniques that serve to make, very precisely, additions, deletions and alterations to the DNA; one of its purposes is to modify specific genes that are responsible for functional alterations in living beings, including humans. Although these techniques are practiced in any living cell (plant or animal), this document discusses exclusively genomic editing in human cells.

Currently, it is feasible to modify some genes responsible for alterations that result in diseases, which is known as gene therapy. However, it is recognized that the feasibility of something must always be subjected to a reflexive review that allows to justify the “why”, “what for” and “when” of something about which we only know the “how” (and only halfway).¹ Being able to do something does not necessarily imply it should be done.

International and national documents relating the human genome

More than 20 years ago, the Convention for the Protection of Human Rights and the Dignity of the Human Being with regard to the Application of Biology and Medicine (Oviedo Convention, April 4, 1997) and the Universal Declaration on the Human Genome and Human Rights of the United Nations Educational, Scientific and Cultural Organization (UNESCO, November 11, 1997) were established. Both documents establish that respect for human dignity is above the applications that could be generated in this regard and restrict human genome modification exclusively for diagnostic or therapeutic reasons “... and only when it is not intended to introduce a modification in the offspring genome” (Article 13 of the Convention).

The General Statute of Health of Mexico considers that the study, research and development of the human genome is a matter of general public health and the Ministry of Health will be in charge of establishing the cases where control is required on this matter, making sure not to limit research freedom (title fifth bis). On the other hand, the Penal Code for the Federal District establishes sanctions for those who, for purposes other than elimination or reduction of serious diseases or defects, manipulate human genes in ways that may alter the genotype (Article 154).

Classification of the use of genetic engineering

Currently, the potential use of these genetic engineering techniques occurs in four contexts that deserve differentiated ethical-regulatory considerations.²

1. *Basic research:* use of genome editing to clarify the mechanism of biological processes in human disease and its treatment. There are established ethical and regulatory provisions to monitor human genome editing through laboratory in vitro models (e.g., registration of research protocols, evaluation and approval of the research process by ad hoc committees, monitoring of results, data presentation to peers, publication of final reports, etc.).

2. *Clinical application in somatic cells*: use of genome modification with the purpose to treat or prevent diseases or disability (e.g., to “correct” cells of different human tissues, except for germ cells). In addition to the provisions mentioned in the previous point, the use in somatic cells should be carried out in the context of clinical trials restricted to the treatment and prevention of disease or disability, with continuous safety and efficacy evaluation, within a context of expected risks/benefits assessment and within a framework of transparency.
3. *Clinical application in germ cells*: this use generates greater concern due to the fact that it is modifying the genome of cells whose changes can be inherited, i.e., human genome-integrated modifications induction and distribution in the population without properly knowing the effects they may have. Greater caution is required in terms of safety and unanticipated effects, since it can importantly impact on human beings from an individual point of view, but also as a species. From the bioethical point of view, germ cells genome modification can affect principles such as autonomy (insofar as the heirs of the modification do not decide on such inclusion in their genome), beneficence/non-maleficence (weighing of the potential benefits against the harm or risks it represents) and, finally, fairness (uneven distribution of benefits, risks and harm among the population, thus unequally affecting or benefiting parts of society). In this sense, international consensus recommends its use exclusively in situations whose only purpose is the treatment or prevention of a serious disease or disability (especially in situations where no other alternative is known), under strict supervision and following very specific criteria.
4. *Human enhancement*: it refers to the use of these technologies to cause cell changes in situations where there is no disease and functional capabilities of the individual are normal, through “human enhancement” (e.g., increased muscle mass to have more strength, increased cognition abilities, esthetic modifications). The use of these technologies outside the context of treatment of diseases or disabilities is currently considered improper, as long as no further information on the risks and potential side effects is available and the impact on the principles of autonomy, beneficence/non-maleficence and fairness cannot be assessed.

The CRISPR-CAS9 system

An important advance in genetic engineering techniques is derived from the description of DNA sequences with “clustered regularly interspaced short palindromic repeats” (CRISPR) originally found in bacteria.³ The RNA produced with these sequences, in conjunction with an enzyme named Cas (cellular apoptosis susceptibility), such as Cas-9,⁴ act as “scissors” to cut DNA at specifically determined sites and constitute that which is known as the CRISPR-Cas9 system, which is more efficient, cheaper and easier to use than other gene editing strategies.

On the other hand, the CRISPR-Cas9 technique has been widely used in the modification of genes in unicellular organisms, plants, non-human animals, somatic human cells and even in human embryos (with no reproductive purposes). The interest aroused by CRISPR-Cas9 in human medicine derives from the possibility to modify immune cells to attack cancer cells and developing treatments to cure genetic diseases such as sickle cell anemia, Huntington’s disease, muscular dystrophy, cystic fibrosis, congenital hypertrophic cardiomyopathy, among others, as well as to create cells that are more resistant to different infections (e.g., infection triggered by the human immunodeficiency virus, HIV).

The Chinese experiment and its scientific-ethical flaws

On November 25, 2018, a video was launched in social networks where researcher He Jiankui (Southern University of Science and Technology in Shenzhen, China) announced the birth “a few weeks prior” of two twin girls who in the embryonic stage underwent gene editing using the CRISPR-Cas9 technology to inactivate the *CCR5* gene.⁵

This *CCR5* gene encodes the CCR5 membrane protein, which is necessary for HIV to enter the CD4 lymphocyte and infect it. The father of the twins is HIV-positive, while the mother is not and both wanted to procreate but feared the possibility of HIV transmission to their offspring. For this reason, they looked for help to assess the possibility of using the in vitro fertilization (IVF) technique with intracytoplasmic sperm injection (ICSI) using washed sperm, to generate an embryo and later transfer it to the mother’s uterus for its gestation.

Dr. He, however, instead of following the recommended procedures for obtaining virus-free embryos,

manipulated them via CRISPR-Cas9 to achieve the modification of the *CCR5* gene. None of the procedures used by He are technically novel. Of course, assisted reproduction techniques such as IVF with ICSI have been clinically used throughout the world for years; furthermore, the sperm washing procedure is widely used to separate and obtain HIV virus-free mobile sperm. There is experience with the CRISPR-Cas9 technique in embryos of various species, even in human embryos. These techniques have barely been evaluated for their potential repercussions and risks in the short and long term, and bioethical discussion therefore revolves around intrauterine transfer of genetically modified embryos. The main identifiable bioethical issues in the “experiment” described by He are the following:

- a) *Unsound experimental design.* One of the problems is that Dr. He experimented in humans (since their embryonic stage), i.e., he transferred human embryos manipulated with the CRISPR-Cas9 technique to the uterus of women for gestation. Technically, this experiment is already within the category of clinical application for reproductive purposes in embryonic cells and contravenes the consensus recommendations published since 2015 by a group of experts in gene editing.⁶ Dr. He reported other gestations, but the data are unknown. The twin girls should be considered as an experiment by themselves and not only as the product of an experiment. The effects of the genetic manipulation they were subjected to must still pass the test of time in terms of the alleged beneficial effects expected for the babies and their future offspring, which only follow-up throughout time will be able to identify. The theoretical benefits (e.g., protection against HIV infection) can only be confirmed by unprotected exposure to HIV infection, which in turn would be bioethically unacceptable.
- b) *That which was offered was not done.* He’s experiment has the problem that it did not correct a genetic defect, but deleted a gene; the twin girls turned out being discordant (one without the two alleles of the gene [complete knockout] and the other with an allele [partial knockout]). People with *CCR5* gene mutations are known to naturally exist in the population and to be resistant to HIV infection, which was the rationale for the manipulation performed by He. However, these mutations may predispose to other infections or increase their severity (e.g., West Nile⁷ or

influenza virus⁸). In addition, in the twin girls experiment, it is unknown if the “gene edition” (or genetic surgery as the involved researchers euphemistically called it) was achieved in all cells of the embryo or only in a certain number (mosaicism) and if the CRISPR-Cas9 effect on these cells occurred only in the position of the *CCR5* gene or if there were other DNA sites subjected to the same effect (off-target effect, which can only be determined by sequencing the entire genome of the babies, which was not done).

- c) *Invalid medical justification.* There is no medical justification about the reasons for conducting the experiment. The reason presented to the parents was “to make their daughters immune to HIV infection”, which is misleading, since the blockage or absence of *CCR5* does not provide immunity, but makes the host not susceptible to infection (the virus cannot enter the cells). In addition, the parents did not require what they were offered, since there are established and highly effective recommendations for generating HIV-free children of infected parents (sperm washing, pre-exposure prophylaxis [highly active antiretroviral therapy]).⁹
- d) *Presentation of the facts in the form of publicity.* Scientific knowledge acquires a progressive value when the data supporting research are subjected to scrutiny by peers, when they are tested and the results are replicated. This is usually made through short communications in conferences and scientific meetings or through publication in scientific journals, after peer review and approval. In this case, the specific experimental data are unknown and no scientific communication has been generated in this regard. He preferred the use of electronic social media (YouTube⁵) in a sensationalist and misleading format.
- e) *Inappropriate informed consent process.* The informed consent process is essential for the participation of human subjects in experimental projects and is based on respect for the autonomy and dignity of people. The consent process would appear to be biased towards the interests of the researcher and be misleading, as it offers the parents something beyond its proven possibilities and hides information about the risks and uncertainty of potential benefits. It is not known if the parents were clearly explained that they were actually making a proxy decision for their

daughters, if they realized that this decision was for life and that it includes their potential offspring. In other words, the future interests of the babies were transgressed without a really informed consent of their parents, who allegedly agreed to the experiment.

- f) *Serious ethical-regulatory offenses.* The protocol registration process appears to have been extremely soft, with little information about what the experiment was really intended for (gestating genetically manipulated embryos) and using a triumphalist language (as they wrote in the protocol approval application: “This is going to be a great science and medicine achievement ever since the IVF technology, which was awarded the Nobel Prize in 2010”).¹⁰ The hospital where the embryo transfer and implantation clinical procedure was carried out and where the babies were finally supposed to have been born, published a statement denying their participation and claiming that “the signatures approving the protocol by its Medical Ethics Committee were forged”. All of the above contravenes the principles of transparency, openness and peer review a scientific process is currently committed to, and generates the perception in society that the scientific process is not serious and even fraudulent due to laxity in regulations and to the fact that only economic or media profits are sought.
- g) *Serious non-disclosed conflicts of interest.* Finally, the researcher He has serious conflicts of interest he did not declare, since he is shareholder of seven high-tech genetics companies and legal representative in six more, possibly involved in the experiment.

Impact of the “HE case” on genetic engineering and genome editing technologies

Genome editing using CRISPR-Cas9 and similar techniques is a highly promising tool with high potential to eliminate, control or mitigate various diseases. Being able to cure monogenetic diseases such as Huntington’s disease, Tay-Sachs disease or cystic fibrosis is not only permissible but also desirable. Clinical trials using this technology for the deactivation of the PD-1 protein gene to treat patients with lung, prostate, bladder and kidney cancer have already been carried out.^{11,12} *ApoE* gene edition to control Alzheimer’s disease is being explored, and manipulating the

same CCR5 gene to improve HIV control is being attempted (in somatic cells of patients with HIV, not in embryonic cells). Recently, it was published that this *CCR5* gene is a therapeutic target for nerve regeneration and functional recovery after stroke and traumatic brain injury.¹³

Conclusions

Demonizing scientific research (basic and clinical) due to cases such as the referred one is a negative effect that should be avoided. The analysis of these flaws coincides with others that have been published¹⁴ and is a call of attention for the scientific community to recognize the importance of bioethical aspects in the execution of projects of this nature. Based on the above, and considering that this is a relevant issue that affects science in Mexico, the following final considerations are proposed:

1. Genome editing should be gradually applied, in such a way that it answers to the state of existing scientific evidence. Its use in basic research and its therapeutic use in somatic cells should be allowed and stimulated following existing ethical guidelines, transparently and under the supervision of established and duly registered review structures (research committees, research ethics committees, National Bioethics Commission, etc.).
2. The use in germ cells should only be allowed in the context of the resolution of diseases or serious disability, under special registration and supervision, as well as with a broadly informed consent of the parents, as long as potential risks and short- and long-term side effects and those with transgenerational reach (inheritable genomic modification) are not determined by other means (similar position to others that have been published¹⁵⁻¹⁸).
3. Application of these technologies to human medicine should be carried out in academic institutions with proven capacity (human resources and equipment), with clear lines of research and beyond possible conflicts of interest. The use of gene editing should respond to genuine scientific and medical interest and the researchers involved should be free of potential conflicts of interest, either economic, industrial, political or media notoriety-related.
4. The scientific community has an ethical obligation to be transparent before society and to diffuse the facts and data it confirms, as well as to

explain the methodologies it uses in order to lift scientific culture and make society in general, and decision makers in particular, see the difference that said scientific knowledge has with regard to other knowledge deriving from dogmas, superstitions, traditions and customs.

5. Introduction of public policies and regulatory norms that inhibit or are contrary to scientific and medical activity in the field of genome editing in humans should be avoided, following the above-set forth guidelines.

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